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Contribution of the stringent response and Anr to anaerobic survival of Pseudomonads

Von der Fakultät für Lebenswissenschaften
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zu Braunschweig
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von Annika Steen
aus Braunschweig

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Abbreviations

AHL	N-acylhomoserine lactone
ALA	5-aminolevulinic acid
Anr	anaerobic regulation of arginine deiminase and nitrate reduction
Ap	ampicillin
APS	ammonium peroxidsulfate
ATP	adenosine triphosphate
BLASTN	basic local alignment search tool for nucleic acid sequences
BLASTP	basic local alignment search tool for protein sequences
bp	base pair
° C	degrees Celsius
Cb	carbenicillin
cDNA	complementary DNA
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CFU	colony forming unit
Cy3/5	cyanine 3/5
(d)dNTP	(di)desoxyribonucleotide triphosphate
dH_2O	deionized water
DksA	DnaK suppressor protein A
DMSO	dimethylsulfoxid
DNA	desoxyribonucleic acid
DNAse	desoxyribobnuclease
Dnr	dissimilatory nitrate respiration regulator
ds	double stranded
DTT	dithiotreitol
EDTA	ethylenediamine tetraacetic acid
et al.	*et alteri* (and others)
e. g.	*exempli gratia* (for example)
e-value	expect value
FC	fold change
fig.	figure
Fis	Factor for inversion stimulation
FRT	Flp recombinase target
fwd	forward

g	earth gravity during centrifugation
g	gram
GC content	guanosine and cytosine content
GLIMMER	Gene Locator and Interpolated Markov Model ER
Gm	gentamicin
h	hour(s)
i. e.	*id est* (that is)
IHF	integration host factor
kb	kilo base pairs
L	liter
λ	wavelength
LB	Luria Bertani
LPS	lipopolysaccharide
m	milli
M	molar (mol/L)
µ	micro
Mb	mega base pairs
MIC	minimal inhibitory concentration
MilliQ	MilliQ water
min	minute(s)
mRNA	messenger RNA
n	nano
NaOH	sodium hydroxide
NAP	nitrate reductase (periplasmatic)
NAR	nitrate reductase (membrane-bound)
NIR	nitrite reductase
nm	nanometer
NOR	nitric oxide reductase
NOS	nitrous oxide reductase
OD	optical density
ORF	open reading frame
ori	origin of replication
PA	*Pseudomonas aeruginosa* gene number
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PM	perfect match

PMT	photo multiplier tube
PP	*Pseudomonas putida* gene number
ppGpp	guanosine tetraphosphate
pppGpp	guanosine pentaphosphate
PQS	Pseudomonas quinolone signal
PST	*Pseudomonas stutzeri* gene number
PRODORIC	prokaryotic database of gene regulation
p-value	probability value
QRT-PCR	quantitative real-time polymerase chain reaction
r	resistance
rev	reverse
RIN	RNA integrity number
RMA	robust multichip average
RNA	ribonucleic acid
RNAse	ribonuclease
RNAP	ribonucleic acid polymerase
rpm	revolutions per minute
rRNA	ribosomal RNA
RT	room temperature
s	second(s)
ss	single strand
SAP	shrimp alkaline phosphatase
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SHX	serine hydroxamate
Sm	streptomycin
tab.	table
TAE	tris acetate EDTA
TBE	tris borate EDTA
Tc	tetracycline
TCA	tricarboxylic acid cycle
TEMED	tetramethylen diamine
Tp	trimethoprim
tris	tris-(hydroxymethyl)-aminomethane
tRNA	transfer RNA
U	unit
UV	ultraviolet

V	volt
vs.	versus
v/v	volume per volume
wt	wild type
w/v	weight per volume

1. Introduction

1.1 The genus *Pseudomonas*

Bacteria of the genus *Pseudomonas* belong to the Gram-negative γ-proteobacteria. They are rod-shaped, polarly flagellated and grow at temperatures in the mesophilic range. Bacteria of the genus *Pseudomonas* have either an obligate aerobic or facultative anaerobic energy metabolism. A striking property is their ability to utilize a variety of carbon sources, e. g. sugars, fatty acids, alcohols, glycols, aromatic compounds, amines or amino acids (Palleroni, 1992). Glucose is metabolized via the Entner-Dourdoroff pathway, which is also called KDPG pathway due to the formation of intermediate 2-keto-3-deoxygluconic acid 6-phosphate (Lessie & Phibbs, 1984).

Members of the genus *Pseudomonas* are found in a variety of ecological niches like water, soil or eukaryotic hosts (Stover *et al.*, 2000). Some bacteria within the genus, such as *Pseudomonas syringae* or *Pseudomonas aeruginosa,* are pathogens or opportunistic pathogens. Others, such as *Pseudomonas fluorescens* or *Pseudomonas putida*, are apathogenic strains.

1.1.1 *Pseudomonas aeruginosa*

P. aeruginosa is a facultative anaerobe with a genome size of 6.3 Mb, a GC content of 68 % and 5671 currently predicted open reading frames (ORF's) (Winsor *et al.*, 2009). The bacterium is a well known opportunistic pathogen for plants, animals and humans (Rahme *et al.,* 1995). As it causes a variety of infections, particularly in predisposed immunocompromised patients, the bacterium is a great problem in clinical environments (Van Delden & Iglewski, 1998). An increasing occurrence of multiresistant *P. aeruginosa* isolates aggravates the therapy of these infections (Hassett *et al.*, 2009).

1.1.1.1 *Pseudomonas aeruginosa* as an opportunistic human pathogen

P. aeruginosa can be isolated from burn wounds, urinary tract or eye infections (Stryjewski & Sexton, 2003). However, the bacterium causes the most severe problems by infecting respiratory tracts of patients suffering from cystic fibrosis (CF). CF is an autosomal-recessive hereditary disease caused by a defect in the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR), a chloride-ABC-transporter (Riordan *et al.*, 1989). A defective CFTR results in an increased sodium, chloride and water absorption (O'Sullivan & Freedman, 2009) which leads to the accumulation of dehydrated secretions on the apical surface of airway epithelia. This thick, dehydrated mucus impairs the motility of cilia on the surface of epithelial cells and consequently, contaminants are not

removed from the lung (Worlitzsch *et al.*, 2002). Due to the lack of mucus clearance bacterial infections are likely to occur in the CF lung and ultimately lead to the patients' demise. Colonization with *P. aeruginosa* is responsible for high rates of mortality among CF patients (Campodonico *et al.*, 2008) by causing a strong immune reaction which subsequently leads to irreversible damage of the lung tissues (Ratjen & Döring, 2003). Fig. 1 depicts the different physiologies of a healthy compared to a CF lung.

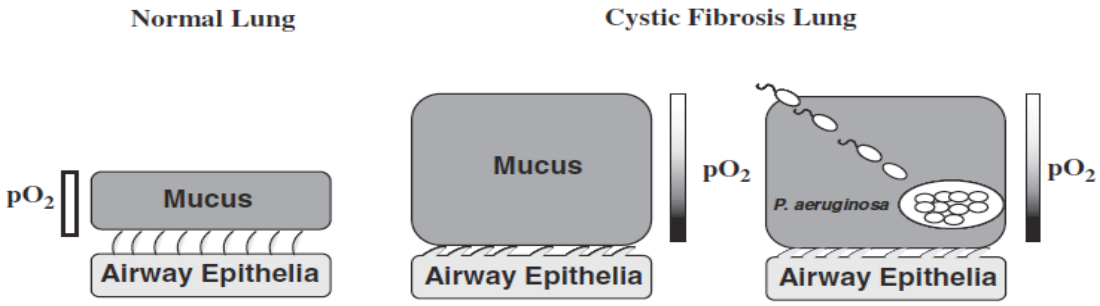

Fig. 1: Physiology of a healthy compared to a CF lung, the latter prone to *Pseudomonas aeruginosa* infections (adapted from Williams *et al.*, 2007). In healthy individuals a thin, hydrated and aerated mucus layer is readily cleared by cilia of airway epithelia. In lungs of patients suffering from CF a thicker mucus layer with low viscosity arises due to an impaired cilial clearance. As a result contaminants are not removed from the lung which allows bacterial colonization. Microaerobic to anaerobic regions in the mucus layer provide a breeding ground for *P. aeruginosa* and other bacteria.

As seen in Fig. 1, an oxygen gradient with microaerobic to anaerobic areas is present in the thick mucus layer of CF patients, which was confirmed by direct measurement of oxygen in CF lung sputum *in situ* (Worlitzsch *et al*., 2002). Additionally, obligate anaerobic bacteria were isolated from sputum samples of a number of CF patients (Rogers *et al.*, 2004). These findings strongly suggest *P. aeruginosa* switches to an anaerobic mode of growth in the CF lung (Hassett *et al.*, 2002). Once *P. aeruginosa* colonizes the airways of CF patients, it attaches to mucus particles and forms biofilm-like microcolonies (Worlitzsch *et al*., 2002).

1.1.1.2 Biofilm growth of *Pseudomonas aeruginosa*

A number of bacteria are able to attach to sur- or interfaces and adapt a sessile lifestyle, resulting in the formation of a biofilm. These are characterized by changes in bacterial physiology, for instance the production of extracellular polymeric substances like lipids, proteins, DNA and polysaccharides, particularly alginate (Moreau-Marquis *et al.*, 2008). In comparison to planktonic growth the biofilm mode of growth offers a number of advantages for the enclosed cells, such as protection from mechanic or chemical attacks

(Mulcahy *et al.*, 2008). Additionally, the bacteria benefit from the production of common goods, such as iron chelating siderophores (Banin *et al.*, 2005) as well as high rates of horizontal gene transfer (Hausner & Wuertz, 1999). However, due to high cell densities within mature biofilm nutrients and oxygen are often limiting, leading to slow growth rates (Mah & O'Toole, 2001). Transcriptome profiles obtained from biofilm cells resemble those of stationary phase rather than exponentially growing cells (Hentzer *et al.*, 2005). Fig. 2 depicts the proposed mechanism leading to the formation of *P. aeruginosa* biofilms.

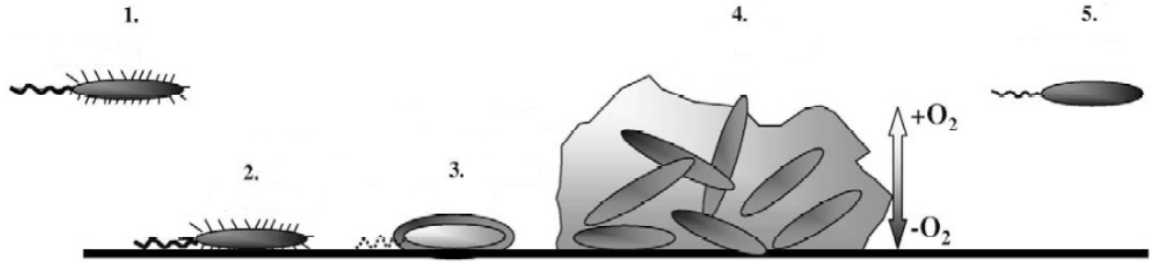

Fig. 2: Proposed model of *Pseudomonas aeruginosa* biofilm formation (adapted from Hassett *et al.*, 2002). Planktonic bacteria (1) attach to a surface by flagella and type IV pili (2). After loss of factors contributing to motility (3), biofilm formation is initiated by exopolysaccharide production. Within the mature biofilm (4), an oxygen gradient develops, leading to the onset of *P. aeruginosa* anaerobic metabolism. Mechanical perturbation causes detachment of cells from the biofilm which subsequently resume a planktonic lifestyle (5).

Biofilm or biofilm-like growth renders bacteria highly tolerant to a number of antibiotics, an effect enhanced by oxygen limitation prevalent in biofilms (Borriello *et al.*, 2004). Although the cells themselves are not more sensitive to antibiotic treatment than planktonic cells, several factors are thought to contribute to antibiotic tolerance of biofilms. These include a reduced metabolic activity and induction of the general stress response due to nutrient and oxygen starvation prevalent in biofilms, an upregulation of genes encoding for efflux pumps, and a poor diffusion through the extracellular matrix of the biofilm (Drenkard, 2003).

A number of factors are involved in bacterial biofilm formation. Requirement of a particular factor often depends on culturing conditions (Kirisits & Parsek, 2006). In *P. aeruginosa*, external signals triggering biofilm formation are e. g. iron limitation (Yang *et al.*, 2007), abundance of nutrients (Heydorn *et al*., 2002) or antibiotic treatment (Hoffman *et al.*, 2005).

Initial biofilm development in *P. aeruginosa* requires a functional quorum sensing network (Davies *et al.*, 1998), *psl* and *pel* operon encoded polysaccharides (Jackson *et al.*, 2004; Friedman & Kolter, 2004), flagellar and twitching motility (O'Toole & Kolter, 1998) and factors contributing to surface attachment, such as fimbrial gene cluster *cup* (Vallet *et al.*, 2001). The Crc (catabolite repression control) protein (O'Toole *et al.*, 2000), transcriptional

regulator GacA (global virulence factor regulator) (Parkins *et al.*, 2001) and response regulator AlgR required for alginate biosynthesis (Whitchurch *et al.*, 1996), were also shown to be involved in *P. aeruginosa* biofilm formation.

Maturation of *P. aeruginosa* biofilms is dependent on cell-to-cell communication via N-acylhomoserine lactones (AHL's). Deletion of the *lasR* gene, mediating the transcriptional response to the presence of AHL's, renders *P. aeruginosa* unable to form a multilayered mature biofilm (Kjelleberg & Molin, 2002). The stationary phase and stress responsive σ-factor σ^S regulates more than 700 genes upon the entry to stationary phase, including many factors of the quorum sensing network (Schuster *et al.*, 2004). Deletion of the σ^S-encoding *rpoS* gene in *P. aeruginosa* resulted in the formation of significantly thicker biofilms (Whiteley *et al.*, 2001; Heydorn *et al.*, 2002), suggesting that σ^S contributes to maintenance of a normal biofilm architecture. The three-component system SadARS (surface attachment defective) involved in regulation of the type III secretion system was also reported to play a role in biofilm maturation (Kuchma *et al.*, 2005). Other factors contributing to maintenance of biofilm architecture and stability, include alginate (Ramsey & Wozniak, 2005), extracellular DNA (Spoering & Gilmore, 2006) and rhamnolipid production (Davey *et al.*, 2003).

A number of signals are known to induce dispersal of a biofilm community. Nutrient limitation has been linked to biofilm detachment in several bacteria, as well as changes in temperature or pH (Gjermansen *et al.*, 2005; Sauer *et al.*, 2004; Thormann *et al.*, 2005). c-di-GMP signaling was implicated to play a role in mediating biofilm disintegration in response to starvation (Gjermansen *et al.*, 2006; Thormann *et al.*, 2006). Changes in oxygen availability apparently have species-specific effects on biofilm dispersal. Biofilms of the facultative anaerobic bacterium *Shewanella oneidensis* were shown to detach upon increased oxygen concentrations (Thormann *et al.*, 2005), whereas *P. putida* cells rapidly dispersed from the biofilm once oxygen becomes limiting (Hansen *et al.*, 2007). EDTA was shown to mediate biofilm dispersal, likely by chelation of divalent cations such as magnesium, calcium, and iron which are required to stabilize the biofilm matrix (Banin *et al.*, 2006). The dispersal process itself is thought to be mediated by increased production of exopolysaccharide lyase and DNase (Allison *et al.*, 1998; Boyd & Chakrabarty, 1994). Also, induction of a Pf4 prophage, which is integrated into the *P. aeruginosa* genome, during biofilm growth (Whiteley *et al.*, 2001), has been shown to result in biofilm dispersal (Rice *et al.*, 2009). Nitric oxide, formed by denitrification during anaerobic conditions (Zumft, 1997), was shown to trigger *P. aeruginosa* biofilm dispersal at concentration which were hot harmful to the individual cell, suggesting that nitric oxide acts as a signaling molecule to trigger dispersal (Barraud *et al.*, 2006).

1.1.2 *Pseudomonas putida*

P. putida is an obligate aerobic bacterium with a genome size of 6.18 Mb, a GC content of 62 % and 5420 currently predicted ORF's (Nelson *et al.*, 2002). Although *P. putida* shares 85 % of the genome with the opportunistic human pathogen *P. aeruginosa* (Bodey *et al.*, 1983), it lacks the key virulence factors which contribute to *P. aeruginosa* pathogenesis (Nelson *et al.*, 2002). The bacterium is commonly found in water and soil habitats, sometimes associated with the surface of plant roots in a mutualistic relationship (Espinosa-Urgel *et al.*, 2002). Due to its metabolic versatility as well as its apathogenic nature, *P. putida* was early considered as a model system for recombinant gene expression.

1.1.2.1 *Pseudomonas putida* as a bioproduction strain

P. putida possesses a very versatile metabolism and is often selectively enriched when more uncommon compounds are offered as carbon or energy sources (Timmis, 2002). Most notably is the bacterium's ability to degrade aromatic hydrocarbons such as toluene (Inoue *et al.*, 1991), benzene (Baldwin *et al*., 2000) or xylene (Phoenix *et al*., 2003), the main compounds of gasoline and profound water pollutants. Additionally, *P. putida* is capable of converting styrene, a precursor of polystyrene foam, into biodegradable polyhydroxyalkanoates (Ward & O'Connor, 2005), which could be useful for elimination of styrofoam waste. Enzymes of these various degradation pathways are often plasmid-encoded (Greated *et al.*, 2002).

KT2440 (Bagdasarian *et al.*, 1981), a plasmid-free derivate of toluene-degrading *P. putida* strain mt-2 (Williams & Murray, 1974), was the first Gram-negative bacterium certified as safety strain by the Recombinant DNA Advisory Committee (Federal Register, 1982). Therefore, *P. putida* is a promising model organism for various biotechnological applications (Molina *et al.*, 1998). For instance, a solvent tolerant *P. putida* S12 strain was constructed for the bioproduction of phenol from glucose (Wierckx *et al.*, 2005). Other S12 derivates have been designed for the production of cinnamic acid from glucose (Nijkamp *et al.*, 2005), or 3-methylcatechol from toluene (Hüsken *et al.*, 2001). Additionally, *P. putida* is suited for heterologous expression of myxobacterial secondary metabolites of medical importance, such as antibiotics or drugs against cancer (Wenzel *et al.*, 2005).

1.2 Adaptation of *Pseudomonas aeruginosa* and *Pseudomonas putida* to oxygen limitation

1.2.1 Adaptation of *Pseudomonas aeruginosa* to oxygen limitation

P. aeruginosa is a facultative anaerobic bacterium. In the absence of the preferred external electron acceptor oxygen, the bacterium can generate energy by anaerobic respiration through the complete reduction of nitrate or nitrite (Davies *et al*., 1989), as well as by fermentation of arginine (Vander Wauven *et al.*, 1984) or pyruvate (Eschbach *et al.*, 2004). Regulation of the anaerobic energy metabolism in *P. aeruginosa* is mediated by global transcriptional regulators Anr, Dnr and the two-component regulatory system NarXL.

1.2.1.1 Denitrification in *Pseudomonas aeruginosa*

In *P. aeruginosa*, the most efficient way to generate energy in the absence of oxygen, is the reduction of nitrogen compounds via denitrification. In this anaerobic respiration, nitrate or nitrite serve as external electron acceptors and are reduced in several steps to nitric oxide, nitrous oxide and finally dinitrogen (Zumft, 1997). Fig. 3 depicts the reduction steps carried out by *P. aeruginosa* during denitrification.

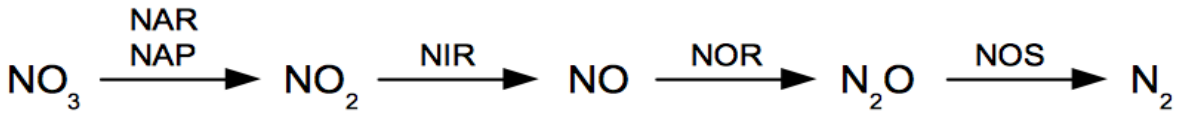

Fig. 3: Reduction of nitrate to dinitrogen during *Pseudomonas aeruginosa* denitrification. Nitrate (NO_3) is converted to nitrite (NO_2) either by membrane-bound nitrate reductase (NAR) or periplasmatic nitrate reductase (NAP), nitrite is converted to nitric oxide (NO) by nitrite reductase (NIR), nitric oxide is converted to nitrous oxide (N_2O) by nitric oxide reductase (NOR), and nitrous oxide is converted to dinitrogen (N_2) by nitrous oxide reductase (NOS).

Compounds necessary for the first step of denitrification, the reduction of nitrate to nitrite, are encoded by the *P. aeruginosa narK$_1$K$_2$GHIJ* gene cluster (Williams *et al.*, 2007). Expression of the genes essential for nitrate reduction is dependent on the oxygen-sensing regulator Anr, the nitric oxide sensing regulator Dnr and the nitrate responsive two-component regulatory system NarXL (Schreiber *et al.*, 2007). Of the two transmembrane antiporters NarK$_1$K$_2$, only NarK$_2$ seems to be essential for nitrate uptake and nitrite efflux (Sharma *et al.*, 2006). Reduction of nitrate to nitrite is catalyzed by dissimilatory nitrate reductase NarGHI (Philippot & Hojberg, 1999). NarI is a membrane-bound cytochrome *b* which transfers electrons from the quinone pool across the

membrane to cytoplasmatic subunit NarH. NarH transfers the electrons via its $[4Fe\text{-}4S]^{2+}$ clusters to the molybdenum cofactor of NarG, which then reduces nitrate to nitrite. NarJ has no catalytic activity but is involved in the assembly of the nitrate reductase (Zumft, 1997).

A second dissimilatory nitrate reductase in *P. aeruginosa*, located in the periplasm, is encoded by genes *napABCDEF* (Williams *et al.*, 2007). Expression of *napABCDEF* was shown to be independent of oxygen, nitrate or nitrite and so far, it is not known which signal induces the operon (Schreiber *et al.*, 2007). The catalytic domains of this second nitrate reductase consist of NapABC, with membrane-bound NapC transferring electrons from the quinone pool to NapB, and NapB passing these electrons to NapA, containing a $[4Fe\text{-}4S]^{2+}$ cluster and a molybdenum cofactor. In contrast to NarGHI, NapABC is unable to translocate protons and as a consequence, no energy is generated. However, it was proposed that NapABC has a role in sustaining the cellular redox balance, which might be important during transition from aerobic to anaerobic growth (Philippot & Hojberg, 1999). Expression of the *napABCDEF* operon was directly repressed by NarL during anaerobic growth, likely to ensure reduction of nitrate via NarGHI (van Alst *et al*., 2009).

The second step of denitrification is catalyzed by nitrite reductase, encoded by the *nirSMCFDLGHJEN* gene cluster, whose expression is regulated by nitric oxide sensing regulator Dnr (Arai *et al.*, 1995 a). Cytochrome cd_1 reductase NirS, located in the periplasm, catalyzes the reduction of nitrite (Silvestrini *et al.*, 1994). NirS consists of two identical subunits, each carrying a heme *c* and a heme d_1 cofactor. Electrons are donated to NirS by NirM, a cytochrome c_{551}, and NirC, a periplasmatic cytochrome *c* (Hasegawa *et al*., 2001), as well as by azurin (Arvidsson *et al.*, 1989). NirFDLGHJE are involved in biosynthesis of heme d_1 (Kawasaki *et al.*, 1995), NirN is a periplasmatic cytochrome *c* with currently unknown function (Hasegawa *et al*., 2001).

Products of the *norBCD* gene cluster, whose expression is induced by nitric oxide sensing regulator Dnr, are required for the reduction of nitric oxide (Arai *et al.*, 2003). NorB, a cytochrome *b*, and NorC, a cytochrome *c*, catalyze the reduction of nitric oxide to nitrous oxide (Arai *et al.*, 1995 b). The exact function of NorD is currently unknown, although it was shown to be essential for *P. aeruginosa* anaerobic growth with nitrate (Arai *et al.*, 1995 b).

In the last step of *P. aeruginosa* denitrification, nitrous oxide is reduced to dinitrogen encoded by the *nosRZDFYL* operon, whose expression is also regulated by nitric oxide sensing regulator Dnr (Arai *et al.*, 2003). Conversion of nitrous oxide to dinitrogen is catalyzed by NosZ, a periplasmatic homodimer containing four copper centers. NosR was shown to be a membrane-bound regulator essential for NosZ activity in *Pseudomonas stutzeri* and it was proposed that NosDFY participate in the insertion of copper into NosZ

(Zumft, 1997).

Fig. 4 depicts a schematic representation of the entire *P. aeruginosa* denitrification pathway (adapted from Zumft, 1997).

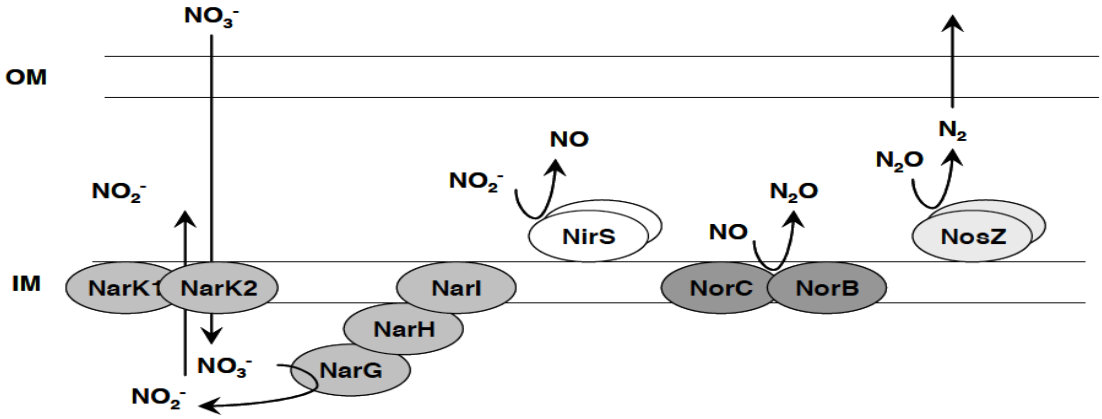

Fig. 4: Schematic representation of the *Pseudomonas aeruginosa* denitrification pathway (adapted from Zumft, 1997). Nitrate is transported into the cell by $NarK_1K_2$, NarGHI reduces nitrate to nitrite. In the periplasm, nitrite is reduced to nitric oxide by NirS, nitric oxide is reduced to nitrous oxide by NorBC and finally, NorZ catalyzes the reduction of nitrous oxide to molecular nitrogen. OM: outer membrane; IM: inner membrane.

1.2.1.2 Fermentative processes in *Pseudomonas aeruginosa*

In the absence of external electron acceptors oxygen, nitrate or nitrite, *P. aeruginosa* is able to grow by the fermentation of arginine, which generates ATP by substrate-level phosphorylation (Vander Wauven *et al.*, 1984). Via the arginine deiminase pathway, arginine is converted to ornithine, carbon dioxide and ammonium, resulting in one molecule ATP. Proteins required for the arginine deiminase pathway are encoded by the *arcDABC* gene cluster. Expression of *arcDABC* is induced by Anr as a result of oxygen limitation (Galimand *et al.*, 1991; Gamper *et al.,* 1991). Also, transcription can be enhanced upon the detection of exogenous arginine by the arginine responsive regulator ArgR (Lu *et al.*, 1999).

Fermentation of arginine begins with the desamination of arginine to citrulline by arginine deiminase ArcA, and subsequent conversion of citrulline to ornithine and carbomoylphosphate by carbomoyltransferase ArcB. Arginine/ornithine antiporter ArcD is involved in exporting the generated ornithine from the cell (Verhoogt *et al.*, 1992). Carbomoylphosphate is converted to carbon dioxide and ammonium by carbamate kinase ArcC, during which one molecule ATP is generated (Williams *et al.*, 2007).

Fig. 5 depicts a schematic representation of the *P. aeruginosa* arginine deiminase pathway (adapted from Vander Wauven *et al.*, 1984).

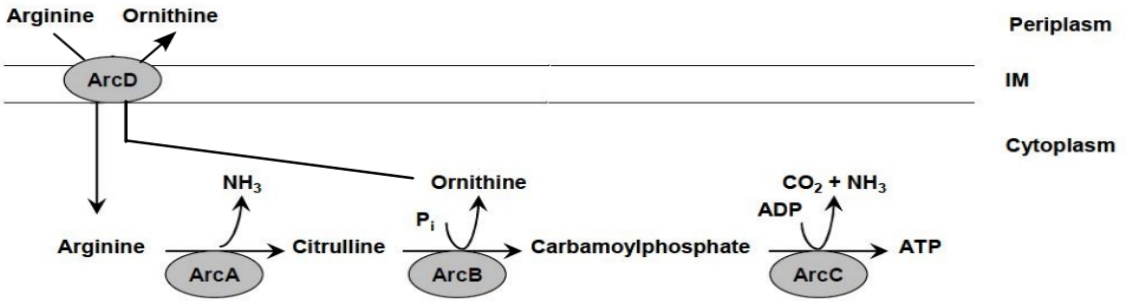

Fig. 5: Schematic representation of arginine fermentation in *Pseudomonas aeruginosa* (adapted from Vander Wauven *et al.*, 1984). Arginine is transported into the cell by ArcD, which also exports subsequently arising ornithine. ArcABC catalyze the conversion of arginine to ornithine, carbon dioxide and ammonium, under generation of one molecule ATP per molecule arginine. IM: inner membrane.

In the absence of external electron acceptors, *P. aeruginosa* is also able to ferment pyruvate, which does not promote growth, but sustains long-term survival (Eschbach *et al.*, 2004). During pyruvate fermentation the substrate is metabolized to succinate, acetate and lactate in different reactions. A simultaneous course of these reactions is required to couple the generation of ATP to a regeneration of NAD^+. Part of the pyruvate fermentation pathway, such as the *ackA-pta* operon encoding for phosphotransacetylase and acetate kinase, is controlled via Anr. Meanwhile, the lactate dehydrogenase encoding *ldhA* gene appears to be constantly expressed. Fig. 6 depicts a schematic representation of *P. aeruginosa* pyruvate fermentation (adapted from Eschbach *et al.*, 2004).

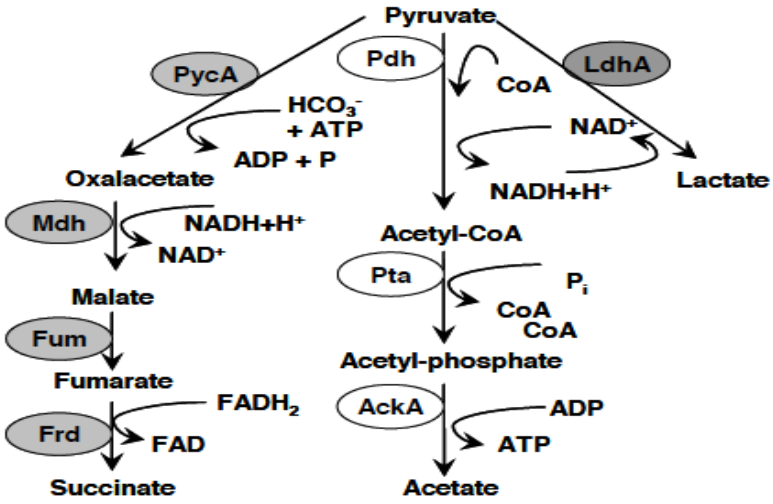

Fig. 6: Schematic representation of pyruvate fermentation in *Pseudomonas aeruginosa* (adapted from Eschbach *et al.*, 2004). Pyruvate is metabolized through a set of different reactions. PycA (pyruvate carboxylase), Mdh (malate dehydrogenase), Fum (fumarase) and Frd (fumarate reductase) metabolize pyruvate to succinate. Pdh (pyruvate dehydrogenase), Pta (phosphotransacetylase) and AckA (acetate kinase) metabolize pyruvate to acetate. Additionally, pyruvate is degraded into lactate by LdhA (lactate dehydrogenase). This step does not generate ATP but is required for recycling of NADH, produced during the conversion of pyruvate to succinate and acetate, to NAD^+.

1.2.2 Regulation of anaerobic metabolism in *Pseudomonas aeruginosa*

Transition from an oxygen-rich environment to oxygen-limiting conditions requires a tight regulatory network to ensure bacterial growth or survival. In *P. aeruginosa*, key regulators involved in this transition are oxygen-sensor Anr (Sawers, 1991), nitric oxide sensor Dnr (Arai *et al.*, 1995 a) and the nitrate responsive two-component regulatory system NarXL (Schreiber *et al.*, 2007).

1.2.2.1 Oxygen sensing regulator Anr

In the regulatory network activated upon anaerobiosis, Anr (anaerobic regulation of arginine deiminase and nitrate reduction) is on top of the cascade. Anr has a 51 % amino acid identity to *E. coli* Crp-Fnr family transcriptional regulator Fnr (fumarate and nitrate reductase regulator) (Sawers, 1991). Fnr detects oxygen via a $[4Fe\text{-}4S]^{2+}$ cluster bound by four N-terminal cysteine residues (Körner *et al.*, 2003). When oxygen concentrations decrease below 10 µM, Fnr dimers are formed which bind conserved DNA-binding sites located approximately 40 bp upstream of the transcriptional start point (Unden *et al.*, 2002). *P. aeruginosa* Anr was also shown to detect oxygen through a $[4Fe\text{-}4S]^{2+}$ center (Yoon *et al.*, 2007) and subsequent binding of conserved DNA sequences (Winteler & Haas, 1996). Fig. 7 depicts a schematic representation of Anr-mediated oxygen sensing in *P. aeruginosa.*

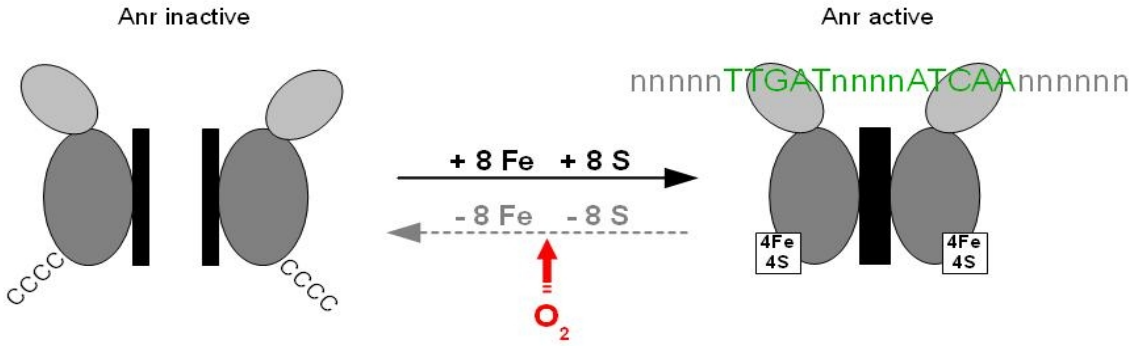

Fig. 7: Proposed model for oxygen-mediated activation and inactivation of transcriptional regulator Anr in *Pseudomonas aeruginosa*. In the absence of oxygen, $[4Fe\text{-}4S]^{2+}$ clusters are assembled which results in the formation of Anr homodimers. If oxygen becomes available, $[4Fe\text{-}4S]^{2+}$ clusters are oxidized, Anr dimers dissolve and are no longer able to act as transcriptional regulators.

P. aeruginosa Anr recognizes conserved promoter regions, the so-called Anr box, with a sequence motif identical to the *E. coli* Fnr-box (Winteler & Haas, 1996). Fig. 8 depicts a sequence logo of the conserved *P. aeruginosa* Anr binding domain generated by the "Virtual Footprint" software on the basis of known Anr binding sites (Münch *et al.*, 2005).

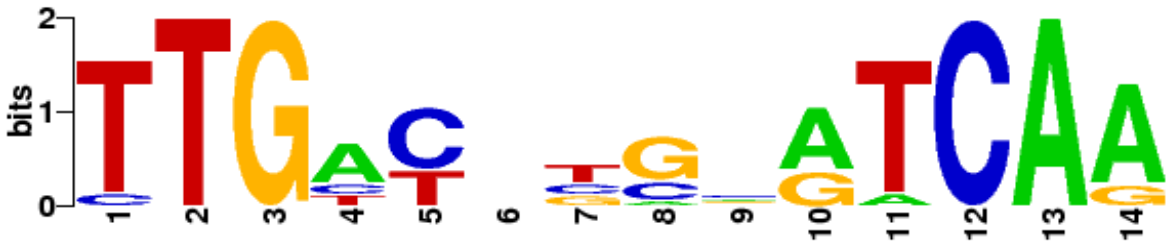

Fig. 8: Sequence logo of *Pseudomonas aeruginosa* PAO1 Anr position weight matrix generated with "Virtual Footprint" (Münch *et al.*, 2005). Information content for a given position is calculated from experimentally determined Anr binding sites and describes the frequency of a base. A sequence logo depicts these results in binary code, in which two bits are required to illustrate the information content of the four letter DNA code. Height of depicted bases indicates the probability of their occurrence at a given position.

Anr directly induces the expression of the *narK$_1$K$_2$GHIJ* operon required for the reduction of nitrate (Schreiber *et al.*, 2007). Furthermore, Anr acts indirectly by inducing genes encoding for Dnr (Arai *et al.*, 1997) and NarXL, which are required for subsequent steps in the denitrification chain (Schreiber *et al.*, 2007). The *arcDABC* operon, required for arginine fermentation, as well as several genes involved in pyruvate fermentation, are also regulated by Anr (Galimand *et al.*, 1991; Gamper *et al.,* 1991; Eschbach *et al.*, 2004).

1.2.2.2 Nitric oxide sensing regulator Dnr

As Anr, *P. aeruginosa* Dnr (dissimilatory nitrate respiration regulator) is a transcriptional regulator of the the Crp-Fnr family (Arai *et al.*, 1997) and exhibits a 25 % amino acid similarity to Anr. Dnr senses nitric oxide rather than oxygen, as it lacks the characteristic N-terminal cysteine residues required for binding of [4Fe-4S] clusters found in Anr or *E. coli* Fnr (Hasegawa *et al.*, 1998). Detection of nitric oxide via Dnr is proposed to act by binding of nitric oxide to an iron-containing heme (Giardina *et al.*, 2008). Although Dnr binds to a nucleotide sequence indistinguishable from the Anr binding motif, promoters of gene *nirS, nirQ*, *norBC* and *nosZ* essential for denitrification are specifically recognized by Dnr (Arai *et al.*, 1999; Arai *et al.*, 2003). However, several genes are regulated by both Anr and Dnr, for instance *hemA*, *hemF*, *hemN* and *narK$_1$K$_2$GHJI* (Krieger *et al.*, 2002; Rompf *et al.*, 1998; Schreiber *et al.*, 2007).

1.2.2.3 Nitrate sensing two-component regulatory system NarXL

Nitrate is detected in *P. aeruginosa* by sensor kinase NarX, which then actives the cognate response regulator NarL (Schreiber *et al.*, 2007). *P. aeruginosa* promoters recognized by NarL possess a specific DNA sequence, similar to the *E. coli* NarL binding site (Krieger

et al., 2002). Although it was shown that induction of *E. coli* NarL results in global changes of the expression profile (Constantinidou *et al.*, 2006), only a few promoters were shown to be regulated by NarL in Pseudomonads so far (Härtig *et al.*, 1999; Krieger *et al.*, 2002; Schreiber *et al.*, 2007; Vollack *et al.*, 1999).

1.2.2.4 Quorum sensing network

Recently, it was shown that AHL's involved in quorum sensing act as repressors of denitrification activity in *P. aeruginosa* (Toyofuku *et al.*, 2007). Pseudomonas quinolone signal (PQS), the third quorum sensing signal in *P. aeruginosa*, also affects denitrification by increasing nitrite reductase activity and simultaneously decreasing nitric oxide reductase activity (Toyofuku *et al.*, 2008).

1.2.3 Adaptation of *Pseudomonas putida* to oxygen limitation

In contrast to *P. aeruginosa*, *P. putida* KT2440 is an obligate aerobic bacterium incapable of respiratory growth under anaerobic conditions. However, in the absence of oxygen some *P. putida* strains are able to grow in liquid cultures supplemented with arginine (Carter *et al*., 1995). Additionally, the bacterium possesses genes encoding for putative enzymes required for other fermentations, such as pyruvate (Nelson *et al.*, 2002). As in *P. aeruginosa*, oxygen concentrations are sensed by transcriptional regulator Anr, which mediates the cellular response for adaptation to anaerobic or microaerobic conditions.

1.2.3.1 Fermentative processes in *Pseudomonas putida*

P. putida possesses an *arcCIAD* gene cluster orthologous to the *P. aeruginosa arcDABC* operon required for anaerobic growth via arginine fermentation (1.2.1.2). *P. putida* KT2440 *arcA* encodes an arginine deiminase required for the potential desamination of arginine to citrulline. Subsequent conversion of citrulline to ornithine and carbomoylphosphate depends on a carbomoyltransferase, which in *P. putida* is encoded by *arcI*. *arcD* encodes an arginine/ornithine antiporter, necessary for export of the generated ornithine from the cell. Lastly, *P. putida* possesses carbamate kinase encoding *arcC*, involved in conversion of carbomoylphosphate to carbon dioxide and ammonium attended by ATP generation.

It was shown that enzymes required for the arginine demininase pathway were induced in *P. putida* when oxygen tension was lowered (Stalon & Mercenier, 1984). However, there are different reports on the bacterium's ability to grow anaerobically via fermentation of arginine. Strains ATCC12633, ATCC25571 and IRC204 did not grow anaerobically on solid medium supplemented with arginine (Vander Wauven *et al.*, 1984), whereas

environmental isolate 2.9 showed weak anaerobic growth in liquid cultures containing arginine (Carter *et al*., 1995).

P. aeruginosa survives in the absence of external electron acceptors for up to three weeks by pyruvate fermentation (1.2.1.2). Several homologs of genes essential for fermentation of pyruvate are also present in the *P. putida* genome (Nelson *et al.*, 2002). Genome annotation revealed ortholog genes encoding for lactate dehydrogenase (*ldhA*) and phosphotransacetylase (*pta*) are present in *P. putida* KT2440. However, the strain lacks an ortholog of the *ackA* gene, encoding an acetate kinase mediating the ATP-generating conversion of acetyl-phosphate to acetate in *P. aeruginosa.*

1.2.4 Regulation of metabolism during oxygen-limiting conditions in *Pseudomonas putida*

Similarly to *P. aeruginosa*, the global oxygen-sensor in *P. putida* is the transcriptional regulator Anr (1.3.2.1) which belongs to the Crp-Fnr family and senses oxygen via [4Fe-4S]$^{2+}$ clusters (Körner *et al.*, 2003). *P. putida* Anr shares 88 % amino acid sequence identity to its ortholog in *P. aeruginosa.* It was shown that *P. putida* Anr regulates expression of several terminal oxidases during oxygen limitation, deletion of the *anr* gene leads to an increased expression of bo_3-type cytochrome as well as cyanide insensitive terminal oxidase and a strong decrease in expression of cytochrome *cbb3-1* terminal oxidase (Ugidos *et al.*, 2008).

1.3 Adaptation to stress and starvation conditions via the stringent response

A decrease in nutrient availability requires a rapid metabolic adaptation of the bacterial cell. The stringent response is a global regulatory system, which mediates major changes in gene expression in response to growth-limiting stress conditions. Key molecule of the stringent response is the alarmone ppGpp, a guanosine nucleotide, and its precursor pppGpp. ppGpp was first discovered as a molecule which accumulated upon nutrient starvation and apparently promoted the downregulation of rRNA promoters in *E. coli* (Cashel & Gallant, 1969). Since then, it was shown for various Gram-negative and Gram-positive organisms, that ppGpp, often in cooperation with co-regulatory protein DksA (DnaK suppressor protein A), severely affects bacterial transcription profiles (Traxler *et al*., 2008; Durfee *et al*., 2008; Eymann *et al.*, 2002; Brockmann-Gretza & Kalinowski, 2006). Induction of the stringent response is primarily aimed at growth inhibition by repression of genes involved in active bacterial growth, such as translation and replication (Chatterji &

Ojha, 2001), but also at the expression of factors involved in stress adaptation (Gustavsson *et al*., 2002; Boes *et al.*, 2008).

1.3.1 Synthesis of ppGpp by proteins RelA and SpoT

In many bacteria cellular ppGpp levels are controlled by the bifunctional protein Rel, which possesses both ppGpp synthetase and hydrolase functions (Jain *et al.*, 2006). Meanwhile, in most γ-proteobacteria bacteria ppGpp levels are determined by proteins RelA and SpoT, first described in *E. coli* (Cashel & Gallant, 1969). While SpoT is a bifunctional protein capable of both synthesizing and degrading ppGpp, RelA lacks an His-Asp motif essential for hydrolase activity and therefore is only capable of ppGpp synthesis (Potrykus & Cashel, 2008). Ribosome-associated protein RelA recognizes stalled ribosomes, which have an uncharged tRNA bound at their A-site due to amino acid starvation (Vidal *et al.*, 1998). The exact mechanism by which RelA is activated is unknown, although it was shown that ribosomal protein L11 seems to regulate RelA catalytic activity (Potrykus & Cashel, 2008).

Several signals inducing SpoT were identified, including phosphate-, carbon-, fatty acid-, iron-, nitrogen-starvation or membrane-perturbing agents (Spira *et al.,* 1995; Xiao *et al.,* 1991; Seyfzadeh *et al.,* 1993; Vinella *et al.,* 2005; Villadsen *et al.,* 1977; Tetu *et al.,*1980). The mechanism leading to activation of SpoT is unknown, although several interactions with other proteins could be determined. For instance, it was shown that *E. coli* SpoT binds the ribosome-associated protein CgtA (Wout *et al.*, 2004) and that the activity of SpoT during fatty acid starvation is regulated by interaction with the acyl-carrier protein involved in fatty acid biosynthesis (Battesti & Bouveret, 2006).

External signals can be used to trigger the stringent response in order to investigate RelA- and SpoT-mediated ppGpp accumulation. The RelA-dependent stringent response can be simulated by the addition of serine hydroxamate (SHX) (Tosa & Pizer, 1971). This L-serine analog inhibits the corresponding serine tRNA synthetase, mimicking serine depletion. The SHX-induced, RelA-mediated stringent response has been thoroughly investigated in various bacteria (Durfee *et al*., 2008; Eymann *et al.*, 2002; Van Delden *et al*., 2001). Recently, it was discovered that alkaline pH values trigger a SpoT-mediated stringent response during anaerobic conditions, possibly resulting from a breakdown of the proton motive force (Boes *et al.*, 2008). Fig. 9 summarizes the effect of RelA and SpoT on cellular (p)ppGpp levels in response to SHX or NaOH treatment, respectively.

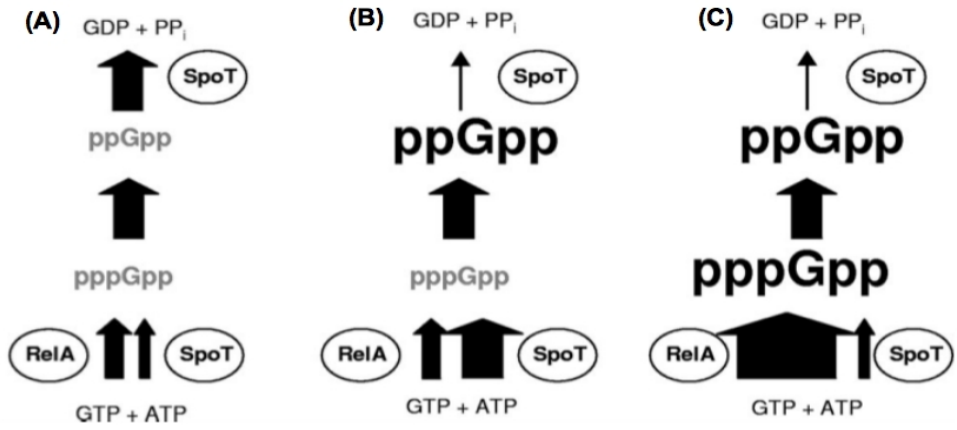

Figure 9: Schematic representation of cellular (p)ppGpp levels during exponential growth, NaOH and SHX treatment (adapted from Boes *et al.*, 2008). Bold arrows indicate increased synthesis or degradation activities, bold letters accumulation of (p)ppGpp. (A) In an exponentially growing culture, synthesized ppGpp is readily degraded by SpoT. (B) In response to NaOH, an increased SpoT synthetase activity and a decrease in hydrolysis rate, result in elevated ppGpp levels. (C) In response to SHX treatment, RelA synthetase activity increases, while SpoT hydrolase activity is reduced, resulting in elevated ppGpp levels. Note that in contrast to NaOH treatment, induction of the stringent response via SHX does not increase the amount of the precursor molecule pppGpp.

As seen in Fig. 9, deletion of genes *relA* and *spoT*, rendering a bacterial strain incapable of synthesizing ppGpp, is of little consequence during normal exponential growth with sufficient nutrient availability. However, once the stringent response is triggered either by nutrient limitation or another stress signal, the absence of both RelA and SpoT hinders an optimal adaptation to the prevalent condition. Deletion of *relA* alone results in decreased ppGpp levels during stationary phase (Xiao *et al.*, 1990), as RelA is the main factor mediating ppGpp synthesis. However, SpoT produces sufficient amounts of ppGpp in a *P. aeruginosa* PAO1 Δ*relA* single mutant strain to induce stringent response regulated genes such as universal stress protein encoding *uspN* (Boes *et al.*, 2008). Only the lack of both *relA* and *spoT* renders Gram-negative bacteria incapable of synthesizing any ppGpp.

1.3.2 Mechanism of action of ppGpp

ppGpp affects transcription by binding to the β- and β'-subunits of the RNA polymerase (RNAP) core enzyme (Toulokhonov *et al.*, 2001), although the exact binding position of ppGpp remains uncertain, and may vary between different bacteria (Tedin *et al.,*1992; Reddy *et al.,* 1995; Chatterji *et al.,*1998; Toulokhonov *et al.*, 2001). Wether transcription is induced or repressed by elevated ppGpp levels depends on the properties of the respective promoters. Target genes repressed by ppGpp typically feature a GC-rich sequence between the -10 region and the transcriptional start site, whereas this region is

AT-rich in promoters which are positively affected (Dalebroux *et al.*, 2010). A transcriptional repression due to a ppGpp-mediated destabilization of RNAP-promoter-complexes (Barker *et al.*, 2001) is particularly effective on intrinsically short-living RNAP-promoter-complexes, for instance on rRNA promoters (Haugen *et al.*, 2006). Meanwhile, a direct positive control of transcription by ppGpp was shown for promoters of genes encoding amino acid biosynthetic enzymes (Paul *et al.,* 2005). Fig.10 summarizes the molecular interactions which result in a direct control of transcription by ppGpp (Dalebroux *et al.*, 2010).

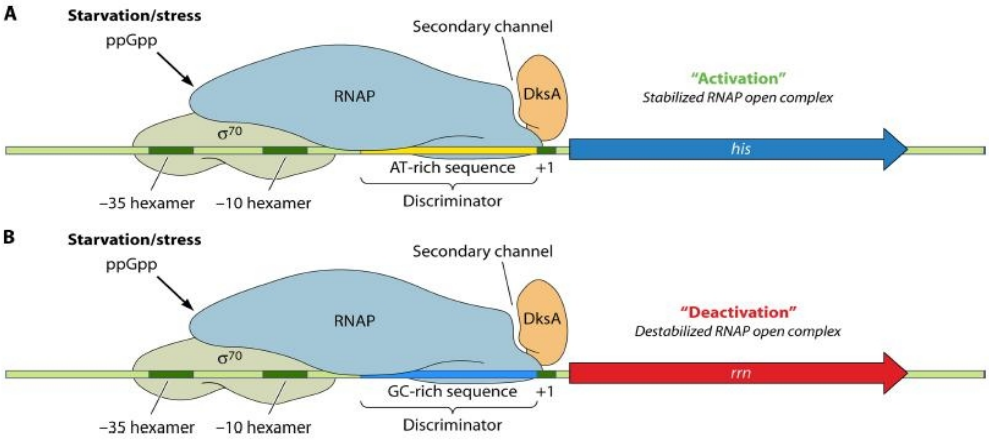

Fig. 10: Schematic representation of transcriptional activation or repression by ppGpp (Dalebroux *et al.*, 2010). In response to stress or starvation conditions, RNAP activity is controlled by ppGpp and its co-regulator DksA (1.3.3). Intrinsic promoter properties determine whether ppGpp induced or represses its target promoters. (A) Activated promoters like that of the E. *coli* histidine biosynthetic (*his*) operon typically have an AT-rich DNA sequence between the -10 region and the transcriptional start site. On these promoters, the binding of ppGpp stabilizes the RNAP open complex. (B) Repressed promoters such as the P1 promoter of rRNA (*rrn*) operons typically have a GC-rich DNA sequence between the -10 region and the transcriptional start site, resulting in a destabilization of the RNAP open complex.

In addition to a direct transcriptional activation, ppGpp can also positively affect transcription by passive and indirect mechanisms. A passive effect is based on an increased RNAP availability due to ppGpp-mediated disintegration of RNAP from strong promoters, such as rRNA promoters, increasing the probability of RNAP to bind other promoters (Zhou & Jin, 1998). Also, increasing amounts of free RNAP offer target molecules for binding of alternate sigma factors. It was shown ppGpp is required for, or increases, transcription from some promoters recognized by *E. coli* σ^{32} (Jishage *et al*., 2006) and σ^{S} (Kvint *et al.*, 2000), as well as *P. putida* σ^{54} (Bernardo *et al*., 2006), σ^{E} (Costanzo & Ades, 2006) and σ^{28} (Österberg *et al.*, 2010). Indirect positive effects of ppGpp on transcription is based on ppGpp-controlled expression of several transcriptional

regulators, which in turn induce their own regulons, leading to the upregulation of various promoters (Durfee *et al.,* 2008).

The accumulation of (p)ppGpp during the stringent response induces a complex transcriptional response, which was shown to affect central biosynthetic pathways, DNA/RNA metabolism, translation, regulation, respiration and transport in *E. coli.* Fig. 11 summarizes the events inducing the stringent response and subsequent cellular processes affected by increased levels of ppGpp (Magnusson *et al*., 2005).

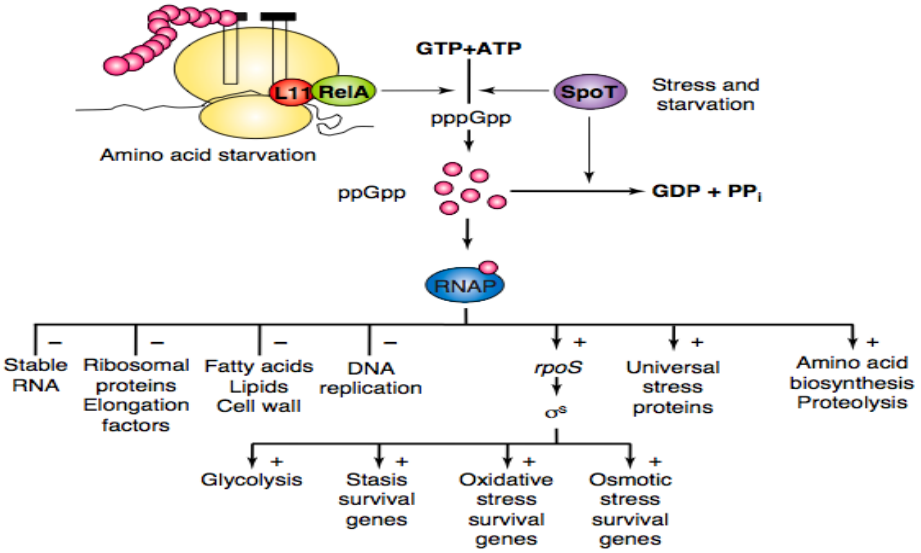

Fig. 11: Overview of ppGpp accumulation and stringent response controlled cellular processes in γ-proteobacteria (Magnusson *et al*., 2005). Proteins RelA and SpoT, activated upon various starvation and stress conditions, synthesize the precursor molecule pppGpp which is then converted to ppGpp. ppGpp interacts with RNAP with direct and indirect effects on transcription profiles. Cellular ppGpp levels are regulated by the hydrolase activity of SpoT.

1.3.3 Co-regulatory protein DksA

Originally identified as a multicopy suppressor of the temperature-sensitive phenotype of *dnaJK* mutants in *E. coli* (Kang & Craig, 1990), DksA (DnaK suppressor protein A) was shown to be involved in a variety of cellular processes, such as quorum sensing (Jude *et al.*, 2003) or virulence (Webb *et al.*, 1999). Recently, it was demonstrated that DksA is required for removal of transcription artifacts, arising upon transcription inhibition in response to amino acid starvation, from DNA strands in order to ensure replication completion (Tehranchi *et al*., 2010).

A number of evidence suggests that DksA acts as a co-regulator of ppGpp-dependent gene regulation. DksA is essential for ppGpp-mediated destabilization of the complex formed by RNAP and rRNA promoters in *E. coli*, which normally leads to down-regulation

of rRNA expression during starvation conditions (Paul *et al*., 2004). For direct activation of promoters of genes encoding for amino acid biosynthetic pathways by ppGpp, DksA is also required (Paul *et al*., 2005). *In vitro* experiments revealed that DksA directly binds to RNAP possibly via a characteristic coiled-coil N-terminus (Perederina *et al.*, 2004).

Although these findings suggest an important role of DksA in cellular adaptation to unfavorable growth conditions via the stringent response, a number of bacteria lack a DksA homolog (Perederina *et al.*, 2004). *E. coli* cells lacking ppGpp and DksA behave similarly in a number of ways, but several findings indicate that DksA and ppGpp also exert opposing effects in the cell. Similar roles of ppGpp and DksA were identified in motility, filamentation and stationary phase morphology of *E. coli* (Magnusson *et al.*, 2007). Also, it was shown that overproduction of DksA compensates the lack of ppGpp and induces ppGpp-dependent promoters in *E. coli*. However, a hyperfimbriated state of an *E. coli* Δ*dksA* mutant strain and a reduced fimbriation of cells lacking ppGpp suggest opposing roles of the stringent response and DksA during some cellular processes (Aberg *et al.*, 2008). Additionally, transcriptome analysis revealed that a subset of genes, including the *fimB* gene involved in fimbriation, showed a different regulation by DksA compared to ppGpp (Aberg *et al.*, 2009).

1.3.4 The stringent response in *Pseudomonas aeruginosa* and *Pseudomonas putida*

Like virtually all known bacteria, *P. aeruginosa* and *P. putida* are capable of synthesizing ppGpp to respond to changes in nutrient availability and other unfavorable growth conditions. Belonging to the group of γ-proteobacteria, Pseudomonads possess proteins RelA and SpoT to carry out the stringent response, as well as the co-regulatory protein DksA.

As the stringent response enables bacteria to cope with environmental changes, its role in the adaptation of *P. aeruginosa* to the CF lung and its contribution to the bacterium's virulence are of great interest. It was shown that RelA overproduction induces *P. aeruginosa rpoS* encoding the σ^S factor as well as the quorum sensing network (Van Delden *et al*., 2001), which are both regulators of a number of *P. aeruginosa* virulence factors (Suh *et al.,* 1999; Sonnleitner *et al.,* 2003; Hogardt *et al.*, 2004). Meanwhile, deletion of the *relA* gene decreased *P. aeruginosa* virulence in a *Drosophila melanogaster* infection model, demonstrating the importance of RelA with regard to pathogenesis *in vivo* (Erickson *et al.*, 2004).

Whereas the RelA-dependent stringent response upon amino acid starvation is well-researched in *P. aeruginosa*, little is known about the stress factors leading to an activation of SpoT. However, recent work demonstrated that alkaline pH values elicit a

SpoT-dependent stringent response which might result from a breakdown of the proton gradient (Boes *et al.*, 2008).

As in *E. coli*, DksA was also shown to be required for ppGpp-dependent downregulation of rRNA promoters in response to amino acid depletion in *P. aeruginosa* (Perron *et al.*, 2005). Also, deletion of the *dksA* gene resulted in a reduced production of quorum-sensing-dependent virulence factors, such as rhamnolipids or LasB elastase (Jude *et al.*, 2003).

In *P. putida* a deeper understanding of the stringent response is of interest as the ppGpp-mediated downregulation of biomass production is an undesirable effect in biotechnological applications. So far, it was shown that ppGpp is required for transcription of the σ^{54}-dependent promoter of the *dmp* operon, encoding for (methyl-)phenol catabolic enzymes (Sze & Shingler, 1999). Meanwhile, stimulation of the Pu promoter on the TOL plasmid pWW0 in *P. putida* mt-2 by 3-methyl benzylalcohol, was not significantly decreased in a ppGpp-deficient strain (Carmona *et al.*, 2000)

1.3.5 The stringent response during oxygen-limiting conditions

Several hints suggest that the stringent response is involved in mediating bacterial adaptation to oxygen limitation, anaerobiosis and the allocation of alternative electron acceptors. It was shown that a *Mycobacterium tuberculosis* mutant incapable of synthesizing ppGpp showed decreased survival rates during long-term anaerobic survival (Primm *et al.*, 2000).

Additionally, a number of genes whose expression was previously shown to be ppGpp-dependent were differentially expressed during anaerobic or microaerobic conditions. Transcriptome analysis carried out to determine *P. aeruginosa* Anr- and Dnr regulons revealed that a number of genes are regulated independently of Anr or Dnr upon anaerobiosis (Trunk *et al.*, 2010). These include a number of genes contributing to growth arrest, resembling stringent response mediated gene expression patterns. Additionally, expression of the *rmf* (ribosome modulation factor) gene which was shown to be regulated by the stringent response in *E. coli* (Izutsu *et al*., 2001), was induced Anr- or Dnr-independently in anaerobically grown *P. aeruginosa* (Trunk *et al.*, 2010).

1.4 Aims of the study

Microbial organisms such as *P. aeruginosa* and *P. putida* often face oxygen-limited to anaerobic environments. Recent research showed that shifting *P. aeruginosa* to anaerobic conditions does not only involve a regulatory network under control of the oxygen-sensing Anr regulator, but in addition involves the stringent response mediated by SpoT, RelA and DksA. However, the impact of the stringent response on anaerobic growth and survival was unclear.

In the first part of this study, the RelA-, SpoT- and DksA-dependent stringent response of *P. aeruginosa* regarding their impact on anaerobic growth and survival should be investigated in detail. Identified growth defects should be further investigated to determine the underlying mechanism. An extensive transcriptome analysis should be applied to investigate the response of *P. aeruginosa* to an elicited stringent response and to define the respective core regulons.

Despite their close relation, *P. aeruginosa and P. putida* differ significantly in their respective energy metabolisms. While *P. aeruginosa* is a facultative anaerobe able to grow by denitrification in the absence of oxygen, *P. putida* is an obligate aerobe. However, both bacteria share almost identical regulatory systems important for oxygen-limited environments, such as the oxygen-sensing regulator Anr and components of the stringent response. In the second part of this work, adaptation of the obligate aerobic bacterium *P. putida* KT2440 to oxygen depletion should be investigated. The Anr regulon of *P. putida* KT2440 should be investigated bioinformatically and experimentally and results compared to existing data of Anr-dependent gene regulation in *P. aeruginosa* PAO1. Also, it should be investigated if the stringent response is involved in mediating anaerobic adaptation of *P. putida* KT2440.

Whereas *P. aeruginosa* is able to grow anaerobically via denitrification, *P. putida* lacks the respective gene clusters mediating the reduction of nitrate to dinitrogen, but it is not known, if additional genes are important for anaerobic growth. Therefore, a comparative genome analysis for identification of genes essential for anaerobic growth should be carried out. In a next step, gene clusters encoding the *P. aeruginosa* denitrification pathway should be transferred into *P. putida* to determine if the bacterium is able to grow anaerobically via nitrate or nitrite reduction. Effects of the transferred denitrification gene clusters on *P. putida* gene expression profiles during anaerobiosis should be monitored by transcriptome analysis.

2. Materials and Methods

2.1 Instruments and Software

application	device	purchaser
Agarose gel electrophoresis	Agagel	Biometra
Agarose gel documentation	Wealtec UV transilluminator	Wealtec
Anaerobic works	Anaerobic work bench	Coy Laboratory Products
Autoclaves	EL3850	Systec
	LVSA 50/70	Zirbu
Biological Safety Cabinet	HeraSafe	Heraeus
Camera	Optio E20	Pentax
Centrifuges	Minispin	Eppendorf
	Biofuge fresco	Heraeus
	Megafuge 1.0 R	Heraeus
	Eppendorf Centrifuge 5424	Eppendorf
DNA sequencing	Genetic Analyzer ABI Prism	Applied Biosystems
Film development	Optimax Typ TR	MS Laborgeräte
Hybridization oven	OV3	Biometra
Incubator	Fine Line	Heraeus
	Thermo Shaker PST-60 HL-4	Lab4You
	Aquatron	Infors
Microarray hybridization	Shel Lab Hybridization Oven	Shel Lab
Microarray scanning	GenePix Personal 4100A	Axon Instruments
	DNA Microarray Scanner	Agilent Technologies
pH determination	Microprocessor pH Meter 211	Hanna Instruments
Pipettes	Eppendorf Research	Eppendorf
PAGE	Mini Protean II	Bio-Rad
RNA quality determination	Agilent 2100 Bioanalyzer	Agilent Technologies
Scales	572	Kern & Sohn
	ALJ 160-4NM	Kern & Sohn
Spectrophotometer	Nanodrop ND-1000-UV/VIS	Peqlab
	Ultrospec	Amersham Biosciencs
Thermocycler	Tpersonal	Biometra
	C1000 Thermalcycler	Bio-Rad
	CFX96 Real-time System	Bio-Rad

Thermomixer	Thermomixer compact	Eppendorf
	Thermomixer comfort	Eppendorf
UV Crosslinker	UV Stratalinker 2400	Stratagene
Vacuum Blot	Vacu-Blot system, pump MP86	Biometra
Vortex	Vortex Genie 2	Scientific Industries
Water purification	MilliQ Synthesis	Millipore

software	**purchaser or distributing website**
2100 Expert	Agilent Technologies
Agilent Scan Control	Agilent Technologies
eArray	earray.chem.agilent.com/earray/
Bioconductor	www.bioconductor.org
BLASTP	www.ncbi.nlm.nih.gov/
CFX Manager V1.1	Bio-Rad
DeVision G	Decon
Feature Extraction 10.7.3.1	Agilent Technologies
GenePix Pro 6.0	Axon Instruments
GLIMMER3	www.cbcb.umd.edu/software/glimmer/
ND-1000 V3.8.2	NanoDrop Technologies
R	www.R-project.org
Primer3Plus	biotools.umassmed.edu/cgi-bin/primer3plus/primer3plus.cgi
PRODORIC	www.prodoric.de
Pseudomonas Genome Database	www.pseudomonas.com
Virtual Footprint	prodoric.tu-bs.de/vfp/
Wikiputida Database	wikiserv.mibi.nat.tu-bs.de/wikiputida/index.php/Main_Page

2.2 Chemicals and Materials

chemicals and enzymes	**purchaser**
dNTP's (for polymerase chain reaction)	New England Biolabs
dNTP's (for reverse transcription)	GE Healthcare
DIG-labeled RNA Molecular Weight Marker II	Roche
GeneChip Labeling Reagent	Affymetrix
GeneRuler DNA Ladder Mix	MBI Fermentas
Hyper Ladder V	Bioline
NeutrAvidin	Invitrogen (Molecular Probes)

Oligonucleotides & Primers	Metabion
One-Phor-All Buffer	GE Healthcare
Random Primers	Invitrogen
rDNase I (RNase free)	usb
Restriction Endonucleases	New England Biolabs
shrimp alkaline phosphatase (SAP)	MBI Fermentas
SsoFast Evagreen Supermix	Bio-Rad
Superase*In (RNAse inhibitor)	Ambion
SuperScript II Reverse Transcriptase	Invitrogen
SYBR Gold Nucleic Acid Gel Strain	Invitrogen (Molecular Probes)
T4 DNA ligase	New England Biolabs
Taq DNA Polymerase	Roche
Terminal Deoxynucleotide Transferase	Promega

Chemicals and reagents not specifically listed here were purchased from the following manufacturers: Amersham Biosciences, Fluka, Merck, Roth, Sigma-Aldrich, Riedel-de Häen.

materials and kits	**purchaser**
24- and 96-Well Plates	Thermo Fisher Scientific
Agilent RNA 6000 Nano Kit	Agilent Technologies
Durapore Membrane Filters (pore size 22 µm)	Millipore
Low 96-Well Clear Plates	Bio-Rad
P. aeruginosa GeneChips	Affymetrix
P. putida Gene Expression Oligo Microarrays	Agilent Technologies
QIAquick Gel Extraction Kit	Qiagen
QIAquick PCR Purification Kit	Qiagen
RNAeasy Mini	Qiagen
ULS labeling Kit with Cy3 & Cy5	Kreatech Biotechnology

2.3 Media and Media Additives

Standard complex medium for *P. aeruginosa, P. putida* and *E. coli* was LB medium (Sambrook & Russell, 2001).

LB medium	10 g	Tryptone
	5 g	Yeast extract
	5 g	NaCl
		in 1 l dH_2O

Standard minimal medium for *P. aeruginosa* and *P. putida* was M9 medium (Sambrook & Russel, 2001), supplemented with trace metals (modified from Schlegel, 2007).

M9 medium	1 x	M9 salts
	2 mM	$MgSO_4$
	0.1 mM	$CaCl_2$
	15 mM	Succinate
	1 x	Trace Metals
		in dH_2O

5 x M9 salts	33.9 g	Na_2HPO_4
	15 g	KH_2PO_4
	2.5 g	NaCl
	5 g	NH_4Cl
		in 1 l dH_2O

Trace Metals	0.148 g	$ZnSO_4$ x 6 H_2O
	0.100 g	$MnCl_2$ x 4 H_2O
	0.236 g	$CoSO_4$ x 7 H_2O
	0.100 g	$NiCl_2$ x 6 H_2O
	0.020 g	$CuCl_2$ x 2 H_2O
	0.050 g	Na_2MoO_4 x 2 H_2O
	1 ml	25 % HCl
		in 1 l dH_2O

For solid media 15 g/l agar were added to the medium. In case of anaerobic growth experiments media were supplemented with either 50 mM KNO_3 or 5 – 10 mM $NaNO_2$. Media for anaerobically grown cultures were introduced to an oxygen-free environment 24 h prior to the experiment to allow all traces of oxygen to diffuse from the media.
Concentrated stock solutions of media additives and antibiotics were dissolved in MilliQ, except for tetracycline, which was dissolved in 50 % ethanol. After preparation stock solutions were sterilized by filtration and used in concentrations described in Table 1.

Table 1: Media additives

	stock solution	concentration for *E. coli*	concentration for *P. aeruginosa*	concentration for *P. putida*
5-aminolevulinic acid	50 mg/ml	5 µg/ml	/	/
carbenicillin	100 mg/ml	100 µg/ml	250 µg/ml	1500 µg/ml
gentamicin	30 mg/ml	10 µg/ml	80 µg/ml	20 µg/ml
sucrose	50 % (w/v)	/	5 % (w/v)	5 % (w/v)
tetracycline	10 mg/ml	5 µg/ml	80 µg/ml	50 µg/ml

2.4 Plasmids and Bacteria

All bacterial strains and plasmids used in this study are listed in Tables 2, 3, 4 and 5.

Table 3: Plasmids used in this study

plasmid	description	reference
mini-CTX-2	Tc^r, self-proficient integration vector	Hoang *et al.* (2000)
pEX18Ap	Ap^r *oriT*$^+$ *sacB*$^+$; gene replacement vector with MCS of pUC18	Hoang *et al*. (1998)
pFLP2	Ap^r; source of FLP recombinase	Hoang *et al*. (1998)
pLAFR3	Tc^r, broad host range cosmid	Staskawicz *et al*. (1997)
pNQ07	Tc^r, pLAFR3 with an insert covering the ATCC17933 genome from ORF PA3858 to approximately PA3880 including the *narK*$_1$*K*$_2$*GHIJ* operon	Quäck, 2005
pPS858	Ap^r Gm^r; source of gentamicin cassette	Hoang *et al*. (1998)
pAS12	Ap^r Gm^r; pEX18Ap with 511 bp fragment upstream of KT2440 *anr,* Gm^r-*gfp* fragment from pPS858 and 533 bp fragment downstream of *anr* between EcoRI and XbaI	this study
pAS14	Ap^r Gm^r; pEX18Ap with 557 bp fragment upstream of KT2440 *spoT*, Gm^r-*gfp* fragment from pPS858 and 513 bp downstream of *spoT* between EcoRI and XbaI	this study
pAS15	Ap^r Gm^r; pEX18Ap with 520 bp fragment upstream of KT2440 *relA*, Gm^r-*gfp* fragment from pPS858 and 563 bp downstream of *relA* between EcoRI and XbaI	this study
pAS29a	Tc^r, pLAFR3 with 26 kb insert covering the ATCC17933 genome from ORF PA05021 to PA0548 including *norBCD* operon	this study

pAS29c	Tc^r, pLAFR3 with 26 kb insert covering the PAO1 genome from ORF PA05016 to PA0545 including *norBCD* operon	this study
pAS36	Tc^r, pLAFR3 with 25 kb insert covering the ATCC17933 genome from ORF PA0507 to PA0529 including *nirSMCFDLGHJEN* and *norBCD* operons	this study
pAS37	Tc^r, mini-CTX-2 with PAO1 P_{nrdJab}-*nrdJab*	this study
pAS39	Ap^r Gm^r; pEX18Ap with 541 bp fragment upstream of PAO1 *nirS*, Gm^r-*gfp* fragment from pPS858 and 600 bp downstream of *nirS* between SacI and HindIII	this study

Table 3: *Escherichia coli* strains used in this study

strain	description	reference
DH10B	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80d*lacZ*M15 Δ*lacX74 deoR recA1 endA1 araD139* Δ*(ara leu)7697 galU galK λ$^-$ rpsL nupG*	Invitrogen
S17λ*pir*	*pro thi hsdR*$^+$ Tp^r Sm^r; chromosome::RP4-2 Tc::Mu-Kan::Tn7λ*pir*	de Lorenzo & Timmis (1994)
ST18	*pro thi hsdR*$^+$ Tp^r Sm^r; chromosome::RP4-2 Tc::Mu-Kan::Tn7λ*pir* Δ*hemA*	Thoma & Schobert (2009)

Table 4: *Pseudomonas aeruginosa* strains used in this study

strain	description	reference
PAO1	wild type	Dunn & Holloway (1971)
PA14	wild type	Rahme *et al*. (2005)
NB159	PAO1 Δ*relA*	Boes *et al*. (2008)
NB170	PAO1 Δ*relA*Δ*spoT*	Boes *et al*. (2008)
PA14 *nirS*::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 589457 (mutant ID: 44650)	Liberati *et al.* (2006)
PA14 *norB*::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 593489 (mutant ID: 25662)	Liberati *et al.* (2006)
PA14 *nosZ*::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 1741334 (mutant ID: 44533)	Liberati *et al.* (2006)
PA14 PA1323::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 4199086 (mutant ID: 31545)	Liberati *et al.* (2006)

PA14 PA1324::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 4198576 (mutant ID: 40121)	Liberati *et al.* (2006)
PA14 PA3729::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 1376957 (mutant ID: 33853)	Liberati *et al.* (2006)
PA14 PA3730::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 1376060 (mutant ID: 37763)	Liberati *et al.* (2006)
PA14 PA3731::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 1375261 (mutant ID: 30418)	Liberati *et al.* (2006)
PA14 PA3732::MrT7	Gm^r, PA14 with insertion of transposon MrT7 at genomic position 1374893 (mutant ID: 39198)	Liberati *et al.* (2006)
PAO1 *pfpI*::IS*lacZ*/hah	Tc^r, PAO1 with insertion of transposon IslacZ/hah at genomic position 399867 (mutant ID: 10045)	Jacobs *et al.* (2003)
PAO1 *pfpI*::IS*phoA*/hah	Tc^r, PAO1 with insertion of transposon ISphoA/hah at genomic position 399593 (mutant ID: 39274)	Jacobs *et al.* (2003)
PAO-MW20	PAO1 Δ*rpoS*, Gm^r	Whiteley *et al*. (2000)
NB163	PAO1 Δ*relA* Δ*spoT* with Gm^r-*gfp* fragment not excised from the *spoT* locus	Nelli Boes
NB224	PAO1 Δ*dksA*	Nelli Boes
NB225	PAO1 Δ*relA*Δ*spoT*Δ*dksA*	Nelli Boes
DE06	PA01 Δ*himD*	Daniela Evers
AS95	PAO1 Δ*nirS*	this study
AS96	PAO1 Δ*relA*Δ*spoT*Δ*nirS*	this study
AS102	PAO1 Δ*dksA*Δ*nirS*	this study
AS103	PAO1 Δ*relA*Δ*spoT*Δ*dksA*Δ*nirS*	this study
AS130	putative suppressor mutant strains of NB170 (PAO1 Δ*relA*Δ*spoT*) isolated from anaerobically grown colony biofilms	this study
AS131	putative suppressor mutant strains of NB224 (PAO1 Δ*dksA*) isolated from anaerobically grown colony biofilms	this study
AS132	putative suppressor mutant strains of NB170 (PAO1 Δ*relA*Δ*spoT*Δ*dksA*) isolated from anaerobically grown colony biofilms	this study

Table 5: *Pseudomonas putida* strains used in this study

strain	description	reference
KT2440	wild type	Bagdasarian *et al.* (1981)
AS32	KT2440 Δ*anr*	this study
AS39 (KT2440-NAR)	Tc^r, KT2240 with cosmid pNQ07	this study
AS40 (KT2440-pLAFR3)	Tc^r, KT2240 with cosmid pLAFR3	this study
AS53	KT2440 Δ*relA*Δ*spoT*	this study
AS86 (KT2440-NIR-NOR)	Tc^r, KT2240 with cosmid pAS36	this study
AS89	Tc^r, KT2440 with integrated pAS37 (mini-CTX-2 with PAO1 P_{nrdJab}-*nrdJab)* after Flp recombinase treatment and cosmid pNQ07	this study
AS90	Tc^r, KT2440 with integrated mini-CTX-2 after Flp recombinase treatment and cosmid pNQ07	this study
AS91	Tc^r, KT2440 with integrated pAS37 (mini-CTX-2 with PAO1 P_{nrdJab}-*nrdJab)* after Flp recombinase treatment and cosmid pAS36	this study
AS92	Tc^r, KT2440 with integrated mini-CTX-2 after Flp recombinase treatment and cosmid pAS36	this study

2.5 Microbiological Techniques

2.5.1 Sterilization

Media and temperature stable solutions were autoclaved at 121 °C and 1 bar positive pressure for 20 min. Temperature sensitive solutions and substances were sterilized by filtration (0.2 µm pore width).

2.5.2 General growth conditions

In all experiments *P. aeruginosa* and *E. coli* were grown at 37 °C, *P. putida* at 30 °C. *E. coli* ST18 was grown in the presence of 5-aminolevulinic acid (ALA) to complement the *hemA* deletion which renders the strain incapable of synthesizing its own ALA (Schobert & Thoma, 2009).

Planktonic growth of aerobic cultures was carried out using Erlenmeyer flasks shaking at

200 rpm. Planktonic growth of anaerobic cultures was carried out using oxygen-impermeable sealed serum flasks shaking at 100 rpm, growth media were supplemented with either nitrate or nitrite as electron acceptor. All cultures were inoculated with an over night culture to an OD_{578} of 0.05. Anaerobic shift experiments were carried out by growing an aerobic culture up to an OD_{578} of 0.5, and transfer of the culture to oxygen-impermeable sealed serum flasks. Addition of nitrate or nitrite depended on the experimental setup and is indicated.

Colony biofilm experiments were carried out by preparing a 1:2500 dilution of an over night culture, to achieve 10^4 to 10^5 cells / membrane filter. 50 µl of the dilution were dispensed on sterile polyvinylidene fluoride membrane filters resting on agar plates (Anderl *et al*., 2000).

Individual growth experiments, applied media and incubation times are indicated in 'Results and Discussion'.

2.5.3 Determination of cell density

The cell density of liquid cultures was determined by measuring the OD at a wavelength of 578 nm. For bacterial strains used in this study, an OD_{578} of 1.0 corresponds to approximately 1 x 10^9 cells / ml.

2.5.4 Determination of colony forming units

Colony forming units were determined by taking an aliquot of the sample and preparation a dilution series in PBS buffer. Appropriate dilutions were plated on LB agar plates and incubated for 16 to 24 h at 37 °C. Afterwards visible colonies were counted and the colony forming unit (cfu) / ml was calculated.

PBS buffer	137	mM	NaCl
	2.7	mM	KCl
	10	mM	Na_2HPO_4
	2	mM	KH_2PO_4
			in dH_2O

2.5.5 Storage of bacterial strains

Glycerol stocks were prepared for long-term storage. For this purpose 850 µl of overnight cultures were mixed with 150 µl of sterile 100 % glycerol. Stocks were frozen and stored at – 80 °C.

2.5.6 Determination of minimal inhibitory concentrations

Minimal inhibitory concentrations (MIC) were determined in 24-well plates in which 5 x 10^5 cells/ml of an overnight culture which were incubated in LB with appropriate antibiotic concentrations. 24-well plates were incubated at 37 °C in a microplate incubator for 20 h. MIC was estimated as the lowest concentration of antibiotics which inhibited growth.

2.6 Molecular Biology Techniques

2.6.1 Determination of nucleic acid concentration and purity

Concentrations and purities of prepared dsDNA, ssDNA and RNA were determined by measuring the absorbance at 230 nm, 260 nm and 280 nm. Absorption at 260 nm of 1.0 corresponded to DNA concentrations of 50 µl/ml and RNA concentrations of 40 µg/ml. Criteria for nucleic acid purity were a ratio of A260 to A280 above 1.8 and of A260 to A230 above 2.0, indicating a low contamination of the sample with proteins and inorganic compounds such as phenol.

2.6.2 Polymerase chain reaction

P. aeruginosa or *P. putida* DNA fragments were amplified by the colony PCR technique. For that purpose a single bacterial colony from agar plate was isolated, resuspended in 20 µl lysis buffer, incubated for 10 min at RT and diluted with 280 µl of MilliQ water.

A 50 µl PCR reaction was prepared using 2.5 µl of DNA template, 5 µl 10 x PCR buffer, 10 pmol of each primer, 200 µm dNTPs, 0.5 U Taq DNA polymerase and 3 % (v/v) DMSO due to the high GC content of *P. aeruginosa* and *P. putida* genomes. An initial denaturation step was followed by 30 x repeated cycles of denaturation, primer annealing and elongation. Annealing temperatures varied according to the primers and were determined in a temperature gradient cycler. Elongation times were chosen based on the length of the desired fragment, with a synthesis rate of Taq DNA polymerase of approximately 1 kb / min.

Preparative PCR reactions were purified using the QIAquick PCR Purification Kit according to manufacturer‘s instructions.

Lysis buffer	60 mM	Tris-HCl, pH 7.8
	1 mM	ETDA
	1 %	SDS

2.6.3 Sequencing of DNA

DNA sequences were obtained with AbiPrism 310 Genetic Analyzer. The required preparatory PCR with fluorescence-labeled ddNTPs and purification of products were carried out as described by the manufacturer. Primers used in the PCR reactions are listed in Table 6.

Table 6: Primers used for sequencing of inserts of pLAFR3-derived cosmids. Forward primer is depicted first and reverse primer second.

region	primer name	sequence (5' → 3' orientation)
40 bp upstream pLAFR3 MCS	M13 for	GTTTTCCCAGTCACGAC
29 bp downstream pLAFR3 MCS	M13 rev	CAGGAAACAGCTATGAC

2.6.4 Electrophoretic separation of DNA

For the analytical separation of DNA fragments, 1 % (w/v in TAE buffer) agarose gels were prepared. Prior to use DNA fragments were mixed with loading dye and GeneRuler DNA Ladder Mix was used as a molecular weight standard according to manufacturer's instructions. Loaded gels were run at 100 V for 30 to 60 min. After electrophoresis the gels were incubated in 0.1 % (v/v) ethidium bromide solution for 10 to 20 min to visualize separated DNA fragments under UV light at 312 nm.

If fragments were intended for further processing, they were extracted from the gel matrix with QIAquick Gel Extraction Kit according to manufacturer's instructions.

TAE buffer (pH 8.0)	40 mM	Tris acetate
	1 mM	EDTA
		in dH_2O

2.6.5 Restriction of DNA

Restriction of DNA was carried out using restriction endonucleases and buffers according to manufacturer's instructions, with incubation times ranging from 4 to 16 h for preparative restrictions, 2 h for analytic restrictions.

2.6.6 Dephosphorylation of DNA

To avoid re-circularization of restricted vector DNA 5' phosphate groups were removed by shrimp alkaline phosphatase (SAP) treatment. 3.5 U SAP were added to the vector and incubated for 1 h at 37 °C. Afterwards SAP was heat inactivated for 15 min at 65 °C and

the vector was purified with QIAquick PCR Purification Kit according to manufacturer‘s instructions.

2.6.7 Ligation of DNA

Ligation of DNA fragments was carried out using T4 DNA ligase and corresponding buffer according to manufacturer's instructions. DNA fragments were incubated for 10 min at 45 °C prior to the ligation reaction to straighten cut DNA ends. 100 ng of vector DNA were mixed with insert DNA in excess and ligation reactions were incubated at 17 °C for 4 to 20 h.

2.6.8 Transformation of calcium chloride competent *Escherichia coli*

E. coli DH10B or ST18 were grown aerobically in 100 ml LB at 37 °C and 200 rpm to an OD_{578} of 0.5. The culture was chilled on ice for 10 min before cells were harvested by centrifugation for 10 min at 4 °C and 4,000 rpm (Heraeus Megafuge 1.0 R). The pellet was resuspended in 10 ml of ice cold $CaCl_2$ buffer and centrifuged as described above. The supernatant was discarded and cells were resuspended in 1 ml of $CaCl_2$ buffer. 50 µl aliquots were prepared, either immediately used for transformation or stored at – 80 °C.
For transformation of $CaCl_2$ competent *E. coli*, cells were first thawed on ice for 15 min and than mixed with 10 to 50 ng of plasmid DNA. After incubation on ice for another 20 min the cells were subjected to a heat shock by incubation at 42 °C for 45 sec. Afterwards 400 µl fresh LB were added for recovery of the cells. After incubation at 37 °C for 30 to 60 min different volumes were streaked on LB agar plates containing the appropriate antibiotic and plates were incubated overnight at 37 °C.

$CaCl_2$ buffer:	100 mM	$CaCl_2$
	10 %	Glycerol
		in dH_2O

2.6.9 Preparation of plasmid DNA

To prepare plasmid DNA alkaline lysis described previously (Sambrook & Russell, 2001) was used. 4 ml of an overnight culture were harvested by centrifugation for 2 min at 4 °C and 13,000 rpm (Heraeus Biofuge fresco). The pellet was resuspended in 300 µl buffer P1. After addition of 300 µl buffer P2 the sample was carefully mixed by inverting and incubated at RT for 5 min. Next, 300 µl of buffer P3 were added, again carefully mixed by inverting and incubated for 5 min at RT. After centrifugation for 20 min at 4 °C and

13,000 rpm (Heraeus Biofuge fresco). 800 µl of the supernatant was mixed with 0.7 volumes of isopropanol and incubated for 10 min at RT. Precipitated plasmid DNA was centrifuged for 15 min at 4 °C and 13,000 rpm (Heraeus Biofuge fresco) before washing with 70 % ethanol. After all traces of ethanol had evaporated, the DNA was solubilized in 50 µl MilliQ and stored at – 20 °C.

Buffer P1	50 mM	Glucose
	25 mM	Tris-HCl, pH 8.0
	10 mM	EDTA, pH 8.0
		in dH_2O
Buffer P2	0.2 N	NaOH
	1 %	SDS
		in dH_2O
Buffer P3	3 M	Sodium acetate, pH 5.3
		in dH_2O

2.6.10 Transformation of *Pseudomonas aeruginosa* and *Pseudomonas putida* by diparental mating

E. coli strain ST18 (Thoma & Schobert, 2009) is a derivate of S17λ*pir* which carries the *tra* genes necessary for conjugation and can be used to transfer plasmids to other bacterial strains. For that purpose overnight cultures of donor strain *E. coli* ST18 and the recipient *Pseudomonas* strain were grown. 1 ml ST18 culture was mixed with 100 µl *P. aeruginosa* or *P. putida* culture and centrifuged for 1 min at 13,000 rpm (Eppendorf MiniSpin). Cells were resuspended in 100 µl LB medium, dropped on an agar plate, dried for 30 min and incubated for 6 h at the recipient strain's optimal growth temperature. During this incubation the mobilizable plasmid is transferred from *E. coli* ST18 to the recipient *Pseudomonas* strain. After the mating procedure cells were scraped off the LB agar plate and resuspended in 1 ml LB medium. Dilutions of the cell suspension were streaked on LB agar plates containing the appropriate antibiotic and incubated at the recipient strain's optimal growth temperature.

2.6.11 Preparation of RNA

Total RNA was prepared by the hot phenol method (Aiba *et al.*, 1981) with minor

modifications. An aliquot of a bacterial culture was mixed with 25 ml ice-cold killing buffer and centrifuged for 10 min at 4 °C and 4,000 rpm (Heraeus Megafuge 1.0 R). After centrifugation cells were shock frozen in liquid nitrogen and stored at – 20 °C for approximately one week.

For RNA isolation cells were resuspended in 125 µl ice-cold sucrose / sodium acetate and gently mixed with 125 µl SDS / sodium acetate. After incubation for 90 sec at 65 °C the suspension was supplemented with 400 µl hot phenol (pH 4.5 – 5.0), mixed vigorously and incubated for 3 min at 65 °C with a brief mixture of the suspension every minute. The suspension then was shock-freezed in liquid nitrogen and spun down for 10 min at RT and 13,000 rpm (Eppendorf MiniSpin). Afterwards the supernatant was again mixed with 400 µl hot phenol (pH 4.5 – 5), incubated for 3 min at 65 °C, shock-frozen and centrifuged for 10 min at RT and 13,000 rpm (Eppendorf MiniSpin). The supernatant was mixed with 400 µl phenol / chloroform / isoamylalcohol and spun down for 2 min at RT and 13,000 rpm (Eppendorf MiniSpin). Incubation with phenol / chloroform / isoamylalcohol was repeated, before the supernatant was mixed with 400 µl chloroform / isoamylalcohol and centrifuged for 2 min at RT and 13,000 rpm (Eppendorf MiniSpin). Afterwards 40 µl 3 M sodium acetate (pH 5.2) and 1 ml 100 % (v/v) ethanol were added to the supernatant for precipitation of RNA. Precipitation was carried out over night at – 20 °C. The precipitated RNA was spun down for 20 min at RT and 13,000 rpm, washed with 70 % (v/v) ethanol.

After all traces of ethanol had evaporated the precipitate was solubilized in 200 µl 10 x DNase I buffer and treated with 3 U RNase-free rDNase I. DNase I digestion was carried out for 30 min at RT. After removal of DNA total RNA was further purified with Qiagen RNAeasy mini kit according to manufacturer's instructions.

Killing buffer	20 mM	Tris-HCl, pH 7.5
	5 mM	$MgCl_2$
		in dH_2O
Sucrose / sodium acetate	300 mM	Sucrose
	10 mM	Sodium acetate, pH 5.2
		in dH_2O
SDS / sodium acetate	2% (w/v)	SDS
	10 mM	Sodium acetate, pH 5.2
		in dH_2O

Phenol / chloroform / isoamylalcohol	50 % (v/v)	Phenol, pH 4.5 – 5.0
	48 % (v/v)	Chloroform
	2 % (v/v)	Isoamylalcohol
Chloroform / isoamylalcohol	96 % (v/v)	Chloroform
	4 % (v/v)	Isoamylalcohol
10 x DNase I buffer	40 mM	Tris-HCl (pH 7.0)
	6 mM	$MgCl_2$

2.6.12 Electrophoretic separation of RNA

For RNA quality check 1 % (w/v in MOPS buffer) agarose gels containing 5 % (v/v) formaldehyde were used. 7 µg of total RNA were mixed with 10 µl RNA loading dye and incubated for 10 min at 65 °C. Samples were loaded onto the gel and electrophoretically separated in 1 x MOPS buffer for 4 to 5 h at 4 °C and 100 V. Gels were stained in 0.1 % (v/v) ethidium bromide solution for 1 h and destained over night in 1 x MOPS buffer. RNA was detected under UV light at 312 nm.

10 x MOPS buffer (pH 7.4)	20 mM	MOPS
	50 mM	Sodium acetate
	10 mM	EDTA
		in dH_2O
RNA loading dye	6.5 ml	**F**ormamide
	1.2 ml	Formaldehyde
	2.0 ml	10 x MOPS
	0.4 ml	50 % (w/v) Sucrose
	350 µM	Bromophenol blue

2.6.13 Quantitative real-time polymerase chain reaction

Quantitative real-time PCR (QRT-PCR) was performed with SsoFast Evagreen Supermix according to manufacturer's instructions. The PCR reaction was recorded with CFX96 Real-Time System and analyzed with Bio-Rad CFX Manager V1.1.

Growth conditions for sampling of bacterial cultures depended on the experiment and are indicated in 'Results and Discussion'. Cells were harvested and total RNA was prepared

(2.6.11). cDNA was used as template for QRT-PCR and generated from 20 µg total RNA. 15 µg random primers and MilliQ water were added to the RNA to a final volume of 16 µl. Primer annealing was performed for 10 min at 70 °C. Afterwards 6 µl 5 x first strand buffer, 3 µl 100 mM DTT, 3 µl dNTP's (25 mM each) 20 U Superase*In were added and the reaction mixture was incubated for 2 min at 25 °C. Subsequent cDNA synthesis was carried out by addition of 600 U SuperScript II and incubation for 2 h at 42 °C. To remove RNA templates after reverse transcription, 10 µl 1 M NaOH and 10 µl 0.5 M EDTA solution (pH 8.0) were added. After 15 min at 65 °C 25 µl 1M HEPES (pH 7.5) and 50 µl 3 M sodium acetate (pH 5.2) were used to neutralize the samples. cDNA was purified with QIAquick PCR Purification Kit according to manufacturer's instructions.

For QRT-PCR 50 ng, 5 ng and 0.5 ng of cDNA were used as a template. All reactions were performed in triplicate, and no template and no reverse transcription controls were carried out in parallel. Standard QRT-PCR programs were carried out with an initial denaturation step at 98 °C for 3.5 min, followed by 40 x repeated cycles of denaturation for 5 sec at 98 °C, primer annealing for 15 sec at 59 °C and elongation for 15 sec at 60 °C. Each cycle was followed by a plate read. Melting curves were generated by a final denaturation step for 10 sec at 98 °C and recorded within the range of 65 – 98 °C.

Primer design was performed with Primer3Plus (Untergasser *et al.*, 2007) according to the following criteria: 100 – 150 bp product length, 18 – 30 bp primer length, 45 – 65 % GC content, 63 – 67 °C primer melting temperature and 65 – 85 °C product melting temperature. Table 7 lists the primers used for detection of target genes PAO1 *narG*, *nirS* and *norB*, and reference genes KT2440 *acpP* and PP5229 with QRT-PCR.

Table 7: Primers used for detection of transcript levels of *Pseudomonas aeruginosa* PAO1 *narG*, *nirS* and *norB,* and *Pseudomonas putida* KT2440 *acpP* and PP5229 with QRT-PCR. Forward primers are depicted first and reverse primers second.

gene	primer name	sequence (5' to 3' orientation)	product size
narG	oAS192	GGCGCTGTAGATGTACCAGGAATAG	137 bp
	oAS193	AGATCTACGTGAAGAACGGCCTGAT	
nirS	oAS194	CGATATCCTTGTAGTTGACCAGCAG	134 bp
	oAS195	CATGACCGTAGACACCCAGACCTAC	
norB	oAS196	CCTGCTGTTCCTGTTCTCCTTCTAC	145 bp
	oAS197	GGTGATCTTCACCAGCACGAAG	
acpP	oAS199	GCGTCAAGGAAGAAGAAGTGACTGT	149 bp
	oAS200	AACGGTAGTGATCTTCTCGGCTTCT	
PP5229	oAS201	GCGTTCCTGTTCGGTGAAGAA	134 bp
	oAS202	GTACCGACGCTGCTCTTGTAGTGTT	

2.7 Construction of *Pseudomonas aeruginosa* and *Pseudomonas putida* knockout mutant strains

To create unmarked gene deletion mutants, the well-established strategies based on *sacB* counter selection and FLP recombinase excision (Hoang *et al*., 1998) was used. In this knockout strategy the target gene is replaced by a gentamicin resistance cassette obtained from vector pPS858. The gentamicin resistance cassette was cloned between to fragments homolog to the upstream and downstream regions of the target gene. This construct was inserted into the MCS of suicide vector pEX18Ap. Tables 8 and 9 depict primers, size of the obtained PCR products and restriction sites used to create knockout constructs used in this study.

Table 8: **Primers and restriction sites used for construction of suicide vectors required for deletion of *Pseudomonas aeruginosa* gene *nirS*.** Restriction sites are underlined, forward primers are depicted first and reverse primers second.

region	primer name	sequence (5' to 3' orientation)	product size	restriction site
upstream of *nirS*	oAS143	CGAGCTCGTCCTGCAACAGCAGGTGAC	541 bp	SacI
	oAS144	CGGGATCCGTCTACAACACCCAGCACGA		BamHI
downstream of *nirS*	oAS129	CGGGATCCCGAGGCGCTCCCTTAGCAGT	600 bp	BamHI
	oAS130	CCCAAGCTTGAACGAAGGCGGCGCCTGCA		HindIII

Table 9: Primers and restriction sites used for construction of suicide vectors required for deletion of *Pseudomonas putida* KT2440 genes *anr*, *relA* and *spoT*. Restriction sites are underlined, forward primers are depicted first and reverse primers second.

region	primer name	sequence (5' to 3' orientation)	product size	restriction site
upstream of *anr*	oAS23	GGAATTCAGCCAGATCGGCGACCTGTA	511 bp	EcoRI
	oAS24	CGGGATCCTGTAGGCCAGTGTGCGCGAT		BamHI
downstream of *anr*	oAS25	CGGGATCCACCTTGGCCTGGCGGTAGAA	533 bp	BamHI
	oAS26	GCTCTAGACTGTCGGCATGCACTTCCAG		XbaI
upstream of *relA*	oAS61	GGAATTCTACGGCGATGATCGAGCAGG	520 bp	EcoRI
	oAS62	CCC AAGCTTCTTCCGTGGCAACCGCACCA		HindIII
downstream of *relA*	oAS63	CCCAAGCTCGGCGTAATCGTACCCCTTG	563 bp	HindIII
	oAS64	GCTCTAGATGTTCGGGCTGGCTCTTCTC		XbaI
upstream of *spoT*	oAS45	GGAATTCATCGTCAGGCTCATGCACTG	557 bp	EcoRI
	oAS46	CCCAAGCTTGTTCACCTCCTGCCGAGGAA		HindIII
downstream of *spoT*	oAS47	CCCAAGCTTTGATCAAGAAGCTGCGTACC	513 bp	HindIII
	oAS48	GCTCTAGAGTAACCTTGCAGAGGAAGGA		XbaI

Assembled suicide vectors were controlled by restriction analysis (2.6.5) and transferred to *P. aeruginosa* or *P. putida* via diparental mating (2.6.10). For selection, the cells were plated on LB agar plates containing gentamicin in a first step. The pEX18Ap plasmid is not able to replicate in *P. aeruginosa,* so growth is only possible if the gentamicin resistance cassette is integrated into the genome. Integration is possible due to homologous recombination between upstream and downstream of the knockout gene present on the suicide vector. A single crossover event leads to integration of the plasmid but not to a deletion of the gene itself. To select strains in which a required double crossover had occurred, clones were selected on LB agar plates containing 5 % (w/v) sucrose and gentamicin. The plasmid pEX18Ap contains the *sacB* gene which encodes a levansucrase, which produces a toxic product by degrading sucrose. Due to this selection, only carbenicillin sensitive clones with integrated gentamicin cassette and removed plasmid can grow.

After a successful knockout the gentamicin resistance cassette was removed from the genome. The resistance cassette from pPS858 is flanked by FRT (Flp recombinase target site) recognition sequences (Hoang *et al*., 1998). Flp-recombinase, which is encoded on the plasmid pFLP2, binds to the FRT recognition sequence and removes the interjacent parts from the genome. pFLP2 was introduced into *E. coli* ST18 by transformation (2.6.8) and then transferred into *P. aeruginosa* or *P. putida* via diparental mating (2.6.10). For selection, the cells were plated first on carbenicillin, allowing Flp recombinase to catalyze the excision of the resistance cassette, which leaves a single FRT sequence of approximately 150 bp. Afterwards, cells were plated on 5 % (w/v) sucrose to remove plasmid pFLP2, which also contains the *sacB* gene, leading to a toxic product when sucrose is present. Only cells which lost the plasmid should grow on sucrose. Finally, the clones were tested on carbenicillin and gentamicin to test if the gentamicin resistance cassette was removed and pFLP2 is eliminated from the strain.

To verify a successful knockout, PCR analysis was carried out (2.6.2). Primers binding upstream and downstream of homologous sequences, listed in Tables 8 and 9, were used for PCR analysis. DNA upstream and downstream of the excised gene were amplified, which should result in a fragment of different size when DNA of the knockout mutant strain was used as a template. As a control, the same PCR was performed with PAO1 or KT2440 wild type.

2.8 Cosmid library screening

A pLAFR3-derived cosmid library covering the *P. aeruginosa* ATCC17933 genome was used to complement anaerobic growth deficiencies of several *Pseudomonas* strains.

Cosmid library and general mating procedures are described elsewhere (Schobert & Görisch, 1999). To isolate cosmids carrying inserts essential for anaerobic growth via denitrification, several recipient strains and conditions were chosen.

In a first approach two *P. aeruginosa* PA14 transposon insertion mutant strains lacking genes encoding nitrite (*nirS*) and nitric oxide reductase (*norB*) respectively, served as recipient strains to isolate *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons. PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains are unable to grow anaerobically with 10 mM nitrite, which was confirmed prior to cosmid library screening. Therefore, these mutants should only grow in the absence of molecular oxygen if respective deletions were complemented by a cosmid carrying the desired genomic region. The ATCC17933 cosmid library was introduced to PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains via diparental mating (2.6.10) under aerobic conditions and the diparental mating was incubated anaerobically for 7 d afterwards. Cosmids of positive clones were isolated by plasmid preparation (2.6.9) and restriction patterns were analyzed via digestion with restriction enzymes EcoRI and HindIII (2.6.5). Finally, the genomic region of the insert was identified by sequencing (2.6.3) using primers which bind the pLAFR3 multiple cloning site (Table 6).

In a second approach *P. putida* KT2440, which is unable to grow anaerobically via denitrification, served as screening tool to isolate the *P. aeruginosa nosDFLRYZ* operon, encoding a nitrous oxide reductase. The cosmid library was screened for complementation of KT2440 anaerobic growth by nitrous oxide reduction. A PA14 *nosZ*::MrT7 transposon insertion mutant strain incubated anaerobically on nitrate incubated in close proximity to the cosmid library screening, was used to generate nitrous oxide. To verify PA14 *nosZ*::MrT7 transposon insertion mutant strain generates sufficient amount of nitrous oxide, *P. stutzeri* was incubated in parallel, as it is able to grow with nitrous oxide as the sole respiratory substrate (Vollack & Zumft, 2001). After 7 d of incubation, small colonies appeared on plates inoculated with *P. stutzeri*, demonstrating that sufficient amounts nitrous oxide was present to promote anaerobic growth by reduction of nitrous oxide.

The ATCC17933 cosmid library was introduced to *P. putida* KT2440 via diparental mating (2.6.10) under aerobic conditions. Afterwards, the diparental mating was plated anaerobically on selective medium and incubated in close proximity to PA14 *nosZ*::MrT7 transposon insertion mutant strain growing on nitrate. Plates were incubated anaerobically for 7 d, followed by a subsequent aerobic incubation for 48 h. Cosmids of positive clones were isolated by plasmid preparation (2.6.9) and restriction patterns were analyzed via digestion with restriction enzymes EcoRI and HindIII (2.6.5). Finally, the genomic region of inserts was identified by sequencing (2.6.3) using primers which bind the pLAFR3 multiple

cloning site (Table 6).

2.9 Transcriptome analysis

2.9.1 *Pseudomonas aeruginosa* Affymetrix GeneChips

RelA-, SpoT- and DksA-dependent expression profiles were investigated by triggering the stringent response during *P. aeruginosa* exponential growth. Experimental procedures were performed by Nelli Bös, transcriptome data analysis was carried out in this study.

Strains Δ*relA*Δ*spoT,* Δ*dksA* and wild type were grown for 5 h in LB supplemented with 50 mM KNO_3. At this time point 3 mM serine hydroxamate (SHX) were added to induce a RelA-dependent starvation signal, 20 mM sodium hydroxide (NaOH) were used to adjust cultures to pH 9.0 and thus triggering a SpoT-dependent stringent response. After 15 min of SHX or NaOH treatment, cells were harvested for transcriptome analysis. All cultures for transcriptome analysis were grown in biological triplicates.

Total RNA was prepared (2.6.11) and reverse transcribed into cDNA for microarray analysis with Affymetrix *P. aeruginosa* GeneChips. cDNA synthesis, fragmentation and labeling were performed according to manufacturer's instructions with minor changes. For cDNA synthesis 10 µg RNA were supplemented with 750 ng random primers and MilliQ water to a final volume of 30 µl. An initial denaturation step for 10 min at 70 °C and incubation for 10 min at 25 °C allowed primer annealing. After primer annealing 12 µl 5 x First strand buffer, 6 µl 100 mM DTT, 3 µl dNTP mix (10 mM each), 30 U SUPERase*In and 1500 U SuperScript II Reverse Transcriptase were added to the reaction. Reverse transcription was carried out by incubation of the reaction mixture for 10 min at 25 °C, 60 min at 37 °C, 60 min at 42 °C and 10 min at 70 °C. Subsequent removal of RNA was carried out by addition of 20 µl 1 N NaOH and incubation for 30 min at 65 °C. Sample neutralization was achieved by addition of 23 µl 1 N HCl. cDNA was purified with QIAquick PCR Purification Kit according to manufacturer's instructions.

Partial digestion of cDNA was carried in 1 x One-Phor-All buffer with 1.1 U rDNase I per µg cDNA for 10 min at 37 °C to yield fragments within the range of 50 – 200 bp. rDNase I was inactivated by heating the sample for 10 min at 98 °C. An aliquot of the sample was separated by PAGE to confirm the generation of the desired fragments. For that purpose 15 % (v/v) polyacrylamide gels were prepared using 3.75 ml polyacrylamid, 2 ml 5 x TBE buffer, 16 µl TEMED, 66 µl 10 % APS and 4.25 ml dH_2O. cDNA samples were mixed with loading dye and loaded to the gel, Hyper Ladder V served as as molecular weight standard. PAGE was carried out at 100 V for 90 to 120 min in TBE buffer. Afterwards gels were stained with 0.1 % (v/v) SYBR gold nucleic stain solution for 10 min at RT. cDNA was detected under UV light at 312 nm.

5 x TBE buffer (pH 8.3)	54 g	Tris
	27.5 g	Boric acid
	20 ml	0.5 M EDTA, pH 8.0
		in 1 l dH_2O

Biotin-ddUTP labeling of fragmented cDNA was archived by incubation with 1 x reaction buffer, 2 µl GeneChip labeling reagent and 60 U terminal transferase for 1 h at 37 °C. The reaction was terminated by addition of 2 µl 0.5 M ETDA (pH 8.0).
Verification of labeling efficiency was carried out by incubating 5 µl of labeled cDNA with 5 µl of the biotin binding reagent NeutrAvidine for 5 min at RT. Afterwards cDNA was separated by PAGE to confirm a successful labeling reaction.

NeutrAvidine	0.4 % w/v	NeutrAvidine
		in 50 mM Tris-HCl (pH 7.0)

Target hybridization, washing, staining and scanning were performed by the Affymetrix Core Facility, Helmholtz Center for Infection Research in Braunschweig. Affymetrix *Pseudomonas aeruginosa* GeneChip microarrays feature oligonucleotide probes of 25 bp length and contains 13 probes of 5549 protein-coding sequences, 18 tRNA genes, a representative of the ribosomal RNA cluster and 199 probes corresponding to 100 intergenic sequences exceeding 600 base pairs.
Raw microarray data was preprocessed with the Bioconductor software framework (Gentleman *et al.*, 2004). Expression values of triplicates were calculated by the robust multichip average (RMA) method using quantile normalization, background corrected perfect match (PM) intensities and median polish as summarization method (Bolstad *et al.*, 2003; Irizarry *et al.*, 2003 a; Irizarry *et al.*, 2003 b). A minimum 2-fold change expression was regarded as biologically significant, data analysis is described elsewhere (3.1.4).

2.9.2 *Pseudmonas putida* Agilent gene expression oligo microarrays

Custom-designed (2.10.3) *P. putida* Agilent gene expression oligo microarrays were used for investigation of various transcriptome profiles.
Culture conditions for transcriptome analysis are described elsewhere (3.2.2.2, 3.2.3.5 and 3.2.3.6), each culture was grown in biological triplicates. Total RNA was prepared (2.6.11) and RNA quality was determined with Agilent 2100 Bioanalyzer and RNA 6000 Nano Kit according to manufacturer's instructions. Samples with a RIN (RNA integrity

number) below 8.0 were not used for microarray analysis.

RNA samples were labeled using ULS labeling kit with Cy3 and Cy5 according to manufacturer's instructions with minor changes. 1 µg RNA was incubated with 1 µl Cy-ULS dye and 1 x labeling buffer in a total volume of 20 µl. The labeling reaction mixture was incubated for 15 min at 85 °C in the dark, as Cy-dyes are sensitive to light. Afterwards the reaction mixture was cooled down on ice and free Cy3 and Cy5 was removed by purification with KREApure columns according to manufacturer's instructions. The degree of labeling was determined by measuring the RNA concentration and the degree of Cy3- or Cy5-labeling in the sample spectrometrically.

300 ng Cy3-labeled and 300 ng Cy5-labeled RNA were joined and incubated with 1 x blocking agent and 1 x fragmentation buffer in a total volume of 25 µl. The fragmentation reaction was carried out for 30 min at 60 °C in the dark. By addition of 25 µl 2 x GE hybridization buffer the fragmentation was stopped. 40 µl of the sample were applied to the microarray and hybridization was carried out for 17 h at 65 °C and 10 rpm. Afterwards the hybridization chamber was disabled in GE wash buffer 1, washed for 1 min at RT in GE wash buffer 1 and for 1 min in GE wash buffer 2 pre-warmed to 37 °C. The gene chip was dried carefully and immediately scanned. Microarray scanning devices of two companies were used.

Transcriptome data discussed in chapters 3.2.2.2, 3.2.2.3 and 3.2.2.4 was obtained with GenePix Personal 4100A (Axon Instruments) and the corresponding GenePix Pro 6.0 software. Each microarray was scanned three times with a resolution of 5 µm and an Auto-PMT (photo multiplier tube) of 0.05. After scanning, the Agilent microarray list (*.gal-file) was loaded and aligned with the scanned microarray. Spots were analyzed for intensity and potential background signals, first by the software and afterwards manually.

Transcriptome data discussed in chapters 3.2.3.5 and 3.2.3.6 was obtained with with DNA Microarray Scanner (Agilent Technologies) and the corresponding software Agilent Scan Control. Microarrays were scanned with 2 µm resolution to create a 20 bit image. After scanning software Feature Extraction 10.7.3.1 was used to excerpt raw microarray data from images.

Raw microarray data was preprocessed with the Bioconductor software framework (Gentleman *et al.*, 2004). Expression values of triplicates were calculated by the robust multichip average (RMA) method using quantile normalization, background corrected perfect match (PM) intensities and median polish as summarization method (Bolstad *et al.*, 2003; Irizarry *et al.*, 2003 a; Irizarry *et al.*, 2003 b). Transcripts which hybridized to only one of two probes or for which calculated p-values (probability values) were higher than 0.05, were excluded from further analysis. A minimum 2-fold change expression was regarded as biologically significant for experiments described in chapters 3.2.2.2 to

3.2.2.4, a minimum 1.5-fold change expression was regarded as biologically significant for experiments described in chapters 3.2.3.5 and 3.2.3.6. Analysis of obtained transcriptome data is described elsewhere (3.2.2.2 to 3.2.2.4; 3.2.3.5 and 3.2.3.6).

2.10 Bioinformatic analysis

2.10.1 *In silico* promoter and regulon analysis with "Virtual Footprint"

To identify *P. putida* KT2440 promoters under control of the transcription regulator Anr and subsequent comparison to the *P. aeruginosa* Anr regulon, an *in silico* analysis was carried out. Regulons were identified using the "Virtual Footprint – Regulon Analysis" software (Münch *et al.*, 2005) with a single pattern search for position weight matrix (PMW) "Anr_Dnr" of *P. aeruginosa*. The following criteria were applied in the analysis: promoter length 350 bp, sensitivity/threshold 1.0 and core sensitivity/size 0.9 to 5. The search was limited to non-coding intergenic regions.

Single promoters were analyzed with "Virtual Footprint – Promoter Analysis" with a pattern search for position weight matrix (PMW) "Anr_Dnr" of *P. aeruginosa*. The following criteria were applied in the analysis: sensitivity 1.0 and core sensitivity/size 0.9 to 5.

2.10.2 Identification of *Pseudomonas putida* open reading frames with "GLIMMER3"

P. putida transcriptome analysis was carried out with custom-designed Agilent gene expression oligo microarrays. To identify putative ORF's missing in the currently available annotation of the KT2440 genome (Nelson *et al.,* 2002), a genome sequence analysis with GLIMMER3 (Gene Locator and Interpolated Markov Model ER) (Delcher *et al.*, 2007) software was carried out by Boyke Bunk. This software is based on 3-periodic nonhomogenous interpolated Markov models to distinguish between coding and noncoding regions. Default parameters of the provided g3-from-scratch.csh script allowed a maximum overlap of two OFR's of 50 bp and a minimum gene length of 110 bp.

The identified putative ORF's were further investigated by analysis of their genomic context and by a BLASTP search to identify homologous proteins in other bacteria. Based on this results, previously unidentified ORF's were assigned and annotated in the "Wikiputida" database.

2.10.3 Agilent gene expression oligo microarray design for *Pseudomonas putida*

The KT2440 genome deposited in the "Wikiputida" database (2.10.2) was used to design 8 x 15K Agilent gene expression oligo microarrays using the "eArray" software (Agilent

Technologies). For each ORF, including rRNA and tRNA genes, three probes of 60 bp length were calculated. Probes against *P. aeruginosa* PAO1 gene clusters PA3871 – PA3880, PA0509 – PA0527.1 and PA3390 – PA3396 encoding PAO1 nitrate, nitrite, nitric oxide and nitrous oxide reductases, respectively, were also included on the microarray.

2.10.4 Comparative genome analysis with "*Pseudomonas* Genome Database"

Genes which might contribute to anaerobic growth in the genus *Pseudomonas* were identified by comparison of the genomes of obligate aerobic and facultative anaerobic strains were compared *in silico*. For that purpose, the "Comparative Genome Search" function provided by the "*Pseudomonas* Genome Database" was applied. The database was screened for genes which were present in *P. aeruginosa* (strain PAO1) and *P. stutzeri* (strain A1501) but not in *P. entomophila* (strain L48), *P. fluorescens* (strains PfO1, Pf-5 and SBW25), *P. mendocina* (strain ymp), *P. putida* (strains W619, GB-1, F1 and KT2440) and *P. syringae* (strains 1448A, B728a and DC3000). Genes directly involved in denitrification were excluded from further analysis, the remaining genes were classified in four categories according to "*Pseudomonas* Genome Database". Class I covers genes whose function was experimentally demonstrated in *P. aeruginosa;* class II covers genes with high similarities to genes of another organism with an experimentally demonstrated function; class III covers genes with a function proposed based on presence of conserved amino acid motif, structural feature or limited sequence similarity to experimentally studied genes; and class IV covers genes with homologies to previously reported genes of unknown function or with no similarity to previously reported sequences.

3. Results and Discussion

3.1 The *Pseudomonas aeruginosa* stringent response during oxygen limitation

Adaptation of *Pseudomonas aeruginosa* to nutrient limitation and other stress conditions via the stringent response is mediated by an interplay of proteins RelA, SpoT and DksA (1.3). Until recently, investigation of the *P. aeruginosa* stringent response was at large focused on the contribution of RelA-mediated ppGpp synthesis and DksA to virulence (Erickson *et al*., 2004; Jude *et al*., 2003). A ppGpp-dependent regulation of *usp* genes important for survival in anaerobic stationary phase (Boes *et al.*, 2008) as well as the importance of DksA for anaerobic growth (Platt *et al.*, 2008) implicates a role of the stringent response during adaption of *P. aeruginosa* to anaerobic growth or survival.
In this study, *P. aeruginosa* PAO1 Δ*relA*, Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains were characterized regarding their growth phenotypes in the absence of molecular oxygen. Transcriptome profiles are analyzed to define the regulatory network underlying the *P. aeruginosa* stringent response during anaerobic conditions. Stringent response regulated factors potentially contributing to the adaptation of *P. aeruginosa* to oxygen limitation were identified and the respective mutant strains characterized with regard to their anaerobic growth phenotypes.

3.1.1 ppGpp and DksA are essential for *Pseudomonas aeruginosa* anaerobic growth and survival

Decreased stationary phase survival in planktonic cultures of mutants lacking a functional stringent response during aerobic, microaerobic or anaerobic conditions has been reported for various bacteria, including *Mycobacterium tuberculosis* (Primm *et al.*, 2000), *Helicobacter pylori* (Mouery *et al.*, 2006), *E. coli* (Nyström, 1994), *Campylobacter jejuni* (Gaynor *et al*., 2005) and *Mycobacterium tuberculosis* (Primm *et al.*, 2000). In this study, it was investigated whether *P. aeruginosa* PAO1 Δ*relA,* Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains also show impaired stationary phase survival. For that purpose, survival rates of aerobically and anaerobically incubated planktonic cultures were monitored over the course of five days. Fig. 12 depicts anaerobic stationary phase survival of PAO1 Δ*relA,* Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains and wild type in the presence of 50 mM nitrate.

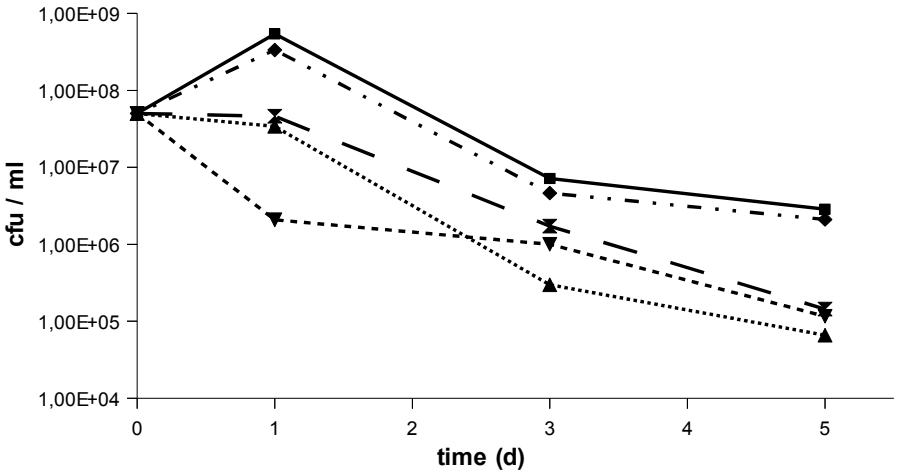

Fig. 12: Anaerobic planktonic survival of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*relA*, Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA*. Cultures of wild type (solid line, squares), Δ*relA* (dotted and dashed line, diamonds), Δ*relA*Δ*spoT* (dashed line, downward triangles), Δ*dksA* (dotted line, upward triangles) and Δ*relA*Δ*spoT*Δ*dksA* (dashed line, hourglasses) were grown anaerobically in sealed serum flasks in LB supplemented with 50 mM KNO_3. Survival rates (cfu/ml) were determined by plating (2.5.4) at indicated time points, values represent the average of two independent experiments.

No decreased stationary phase survival of *P. aeruginosa* mutants affected in their ability to carry out the stringent response was observed during aerobic planktonic growth (data not shown). However, as seen in Fig. 12, PAO1 mutant strains Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* display up to 250-fold reduced survival rates compared to wild type in the absence of oxygen and presence of nitrate. After five days of incubation, approximately 5 x 10^6 cfu/ml were detected for PAO1 wild type, whereas approximately 1 x 10^5 cfu/ml were detected for Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains. No reduced number of colony forming units were observed for a Δ*relA* mutant strain, which behaves similar to PAO1 wild type, indicating SpoT alone is able to control ppGpp levels essential for *P. aeruginosa* stationary survival during anaerobic conditions.
These results demonstrate an importance of ppGpp and DksA for *P. aeruginosa* survival in the absence of molecular oxygen. It has been shown that *P. aeruginosa* switches to an anaerobic mode of growth within the CF lung (Hassett *et al.*, 2002; Worlitzsch *et al.*, 2002; Schobert & Tielen, 2010). Therefore, the stringent response may contribute to the bacterium's pathogenesis due to its importance for anaerobic stationary phase survival.

3.1.2 ppGpp and DksA are essential for *Pseudomonas aeruginosa* anaerobic colony biofilm growth and survival

Upon colonization of the airways of CF patients, *P. aeruginosa* readily attaches to mucus particles and forms biofilm-like microcolonies (Worlitzsch *et al*., 2002). Consequently, a biofilm mode of growth is thought to resemble *in vivo* infection conditions more closely

than planktonic growth. As biofilm growth is accompanied by a number of physiological changes in contrast to planktonic growth (1.1.1.2), anaerobic growth phenotypes of *P. aeruginosa* mutant strains Δ*relA,* Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* were also investigated in a colony biofilm model (2.5.2) in the presence of 50 mM nitrate.

Particularly during the later stages of biofilm growth, cells often face nutrient limitation (1.1.1.2). To mimic these starvation conditions, colony biofilms grown on membrane filters were incubated on a single LB plate over the course of the experiment. In a first experiment, colony forming units of aerobically grown colony biofilms of PAO1 Δ*relA,* Δ*relA*Δ*spoT*, Δ*dksA,* Δ*relA*Δ*spoT*Δ*dksA* and wild type were determined (data not shown). Deletion of *relA* alone had no effect on aerobic colony biofilm growth compared to PAO1 wild type. The Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains displayed slightly reduced number of colony forming units, with an approximately 2.5-fold average reduction compared to wild type levels over the course of five days. However, these results do not implicate a crucial role of ppGpp and DksA in adaptation of *P. aeruginosa* to aerobic biofilm growth on LB (data not shown).

In a next step, colony biofilm growth was monitored during anaerobic conditions in the presence of 50 mM nitrate. Fig. 13 depicts colony forming units of anaerobically grown colony biofilms of PAO1 Δ*relA,* Δ*relA*Δ*spoT* mutant strains and wild type, Fig. 14 depicts colony forming units of anaerobically grown colony biofilms Δ*dksA,* Δ*relA*Δ*spoT*Δ*dksA* mutant strains and wild type.

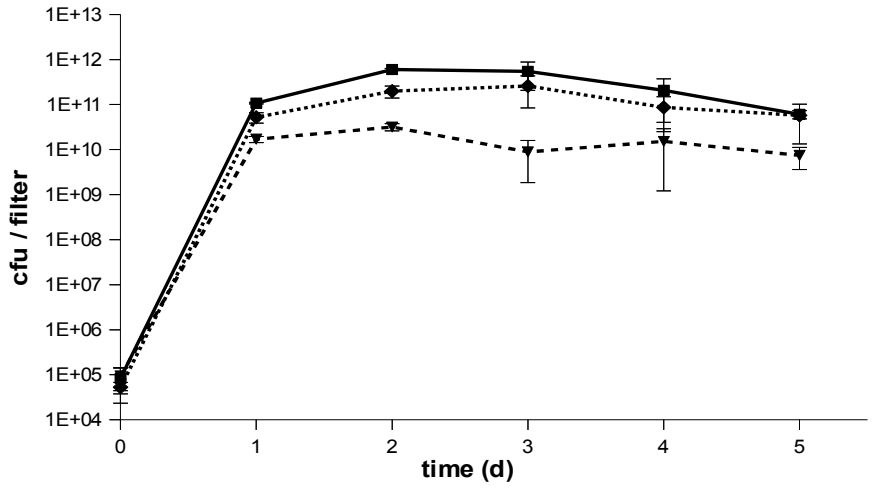

Fig. 13: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*relA* and Δ*relA*Δ*spoT.* Colony biofilms of wild type (solid line, squares), Δ*relA* (dotted line, diamonds) and Δ*relA*Δ*spoT* (dashed line, triangles) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), standard deviations were calculated from three independent experiments.

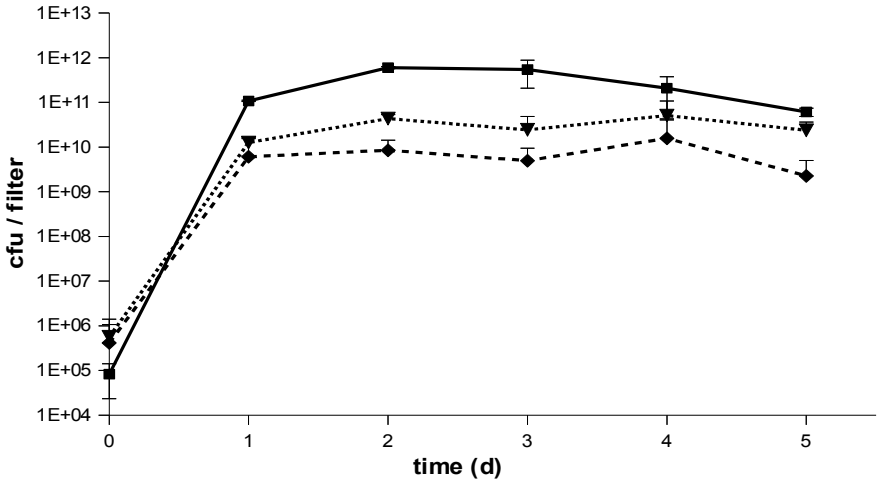

Fig. 14: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA*. Colony biofilms of wild type (solid line, squares), Δ*dksA* (dashed line, diamonds) and Δ*relA*Δ*spoT*Δ*dksA* (dotted line, triangles) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), standard deviations were calculated from three independent experiments.

As seen in Fig. 13, a Δ*relA* single mutant strain displays only slightly reduced anaerobic biofilm growth compared to wild type PAO1. Over the course of five days an average 2.5-fold reduction of colony forming units compared to wild type levels was detected. Meanwhile, a Δ*relA*Δ*spoT* double mutant strain, which does not synthesize any ppGpp and thus is unable to carry out the stringent response, had a severe defect during anaerobic colony biofilm growth on nitrate. For this strain, the number of colony forming units was up to 60-fold reduced in comparison to levels of PAO1 wild type.

As seen in Fig. 14, a *P. aeruginosa* Δ*dksA* mutant strain is also impaired during anaerobic colony biofilm growth. The number of colony forming units was up to 100-fold reduced in comparison to PAO1 wild type, suggesting that DksA is at least equally important for anaerobic colony biofilm growth of PAO1 as ppGpp. Interestingly, the defect during anaerobic colony biofilm growth observed for Δ*relA*Δ*spoT* and Δ*dksA* mutant strains was partly restored in a Δ*relA*Δ*spoT*Δ*dksA* triple mutant strain. As seen in Fig. 14, PAO1 Δ*relA*Δ*spoT*Δ*dksA* mutant strain reaches up to approximately 1×10^{11} cfu/filter, representing only a 10-fold reduction compared to wild type levels. Transcriptome analysis in *E. coli* revealed that a number of genes, whose expression was altered in either Δ*relA*Δ*spoT* or Δ*dksA*, were not differentially regulated in a Δ*relA*Δ*spoT*Δ*dksA* triple mutant strain, although the reason for this observation remains unknown (Aberg *et al.*, 2009).

In addition to a decreased number of colony forming units, anaerobic colony biofilms formed by Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains are also affected in

their morphology compared to wild type (Fig. 15).

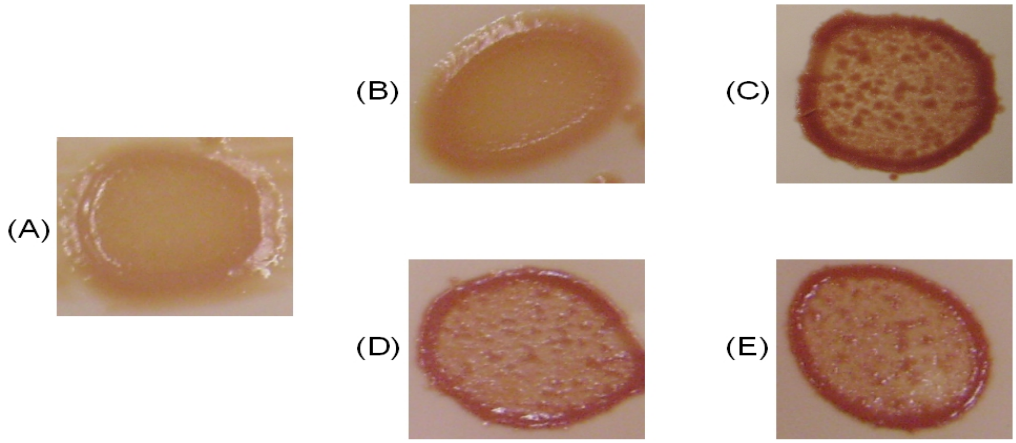

Fig. 15: Morphologies of anaerobically grown colony biofilms of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*relA*, Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA*. Colony biofilms of (A) wild type, (B) Δ*relA*, (C) Δ*relA*Δ*spoT*, (D) Δ*dksA* and (E) Δ*relA*Δ*spoT*Δ*dksA* were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony biofilm morphologies were documented after five days of anaerobic incubation by photography with a Pentax Optio E20 camera.

As seen in Fig. 15, anaerobically grown colony biofilms of PAO1 wild type and Δ*relA* single mutant strain show a smooth, coherent surface. Meanwhile, colony biofilms formed by Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains have a more disrupted, spotty appearance. As expected, phenotypes of aerobically grown colony biofilms of Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains had wild type like morphologies (data not shown).

Cells of anaerobically grown colony biofilms of Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains also displayed a drastic increase in cell length compared to wild type which was not observed during aerobic colony biofilm growth (data not shown). A filamentous phenotype of *E. coli* strains lacking ppGpp has been reported previously (Magnusson *et al.*, 2007; Traxler *et al.*, 2008). Several hints indicate that ppGpp is involved in modulating cell division, which accounts for the enlarged cell morphology of strains lacking a functional stringent response (Schreiber *et al.*, 1995; Vinella & D'Ari, 1994).

During anaerobic colony biofilm growth of Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains, distinct colonies become visible within as well as on the outer rim of the biofilm. In order to investigate if these colonies arise due to formation of suppressor mutants, they were isolated and used to inoculate new anaerobic colony biofilms. Interestingly, colonies isolated from Δ*relA*Δ*spoT* parent strain formed colony biofilms with wild type like morphology during anaerobic conditions, although no increased number of colony forming units was observed (data not shown). Meanwhile, colonies isolated from

Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* parent strains behaved similarly to the respective parent strain with regard to both morphology and number of colony forming units (data not shown). These findings indicate that at least deletion of *relA* and *spoT* may result in the formation of suppressor mutant strains, in which one or more additional mutations arise and compensate effects caused by the absence of ppGpp.

As seen in this chapter, *P. aeruginosa* cells unable to synthesize ppGpp or DksA have a severe growth defect during anaerobic colony biofilm growth in the presence of nitrate, which is of particular interest as biofilm growth resembles *P. aeruginosa in vivo* infection conditions. ppGpp has been previously implicated to positively affect biofilm formation, although in most reports this was demonstrated under aerobic conditions. For instance, an *E. coli* Δ*relA*Δ*spoT* mutant strain was shown to be affected in its ability to form biofilms (Aberg *et al.*, 2008), an effect proposed to be associated with slow-growing conditions (Balzer & MeLean, 2002). Interestingly, an *E. coli* Δ*dksA* mutant showed more rapid biofilm formation during aerobic conditions than the corresponding wild type (Aberg *et al.*, 2008). However, this phenomenon was directly linked to the opposing roles of ppGpp and DksA in regulation of type I fimbriae, which are important for *E. coli* biofilm establishment. In *Streptococcus mutants*, deletion of *relA*, the single gene responsible for ppGpp synthesis and hydrolysis, also resulted in decreased rates of biofilm formation in a 5 % CO_2 atmosphere (Lemos *et al.*, 2004). Surprisingly, a *Campylobacter jejuni* mutant unable to synthesize ppGpp formed biofilms more rapidly than the corresponding wild type strain (McLennan *et al.*, 2008).

In conclusion, the stringent response has been shown to significantly affect biofilm development in various microorganisms, although different biofilm phenotypes have been observed. These differences may be attributed to variations in metabolism, stringent response regulons and biofilm types of the respective bacteria. Likely, a common feature of these biofilms is the occurrence of local microaerobic to anaerobic areas caused by oxygen consumption of bacteria on the biofilm surface (Stewart & Franklin, 2008). Phenotypes described for *S. mutants* and *E. coli* mutant strains unable to synthesize ppGpp may be influenced by the oxygen limitation prevalent within bacterial biofilms. These local anaerobic zones are also present within aerobically grown *P. aeruginosa* colony biofilms and could explain the observed moderate decrease in cell numbers. However, a complete absence of oxygen as well as the presence of nitrate may account for the distinct decrease in the number of colony forming units of anaerobically grown colony biofilms of PAO1 Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains.

P. aeruginosa grows anaerobically by reduction of nitrate or nitrite to dinitrogen (1.2.1.1). An intermediate of these reactions is nitric oxide, which has been reported to mediate *P. aeruginosa* biofilm dispersal in continuous-culture flow cell experiments at concentration

which were not harmful to the individual cell (Barraud *et al.*, 2006). At larger concentrations nitric oxide causes severe damage in biological systems, as it readily reacts with metals within macromolecules, for instance in heme proteins such as cytochromes (Wink & Mitchell, 1998). Indirect nitric oxide induced damaging effects are due to the reaction of nitric oxide with either superoxide or oxygen, yielding reactive nitrogen oxide species (Wink & Mitchell, 1998). In this study, it was investigated whether an accumulation of nitric oxide, due to a potential deregulation of genes involved in *P. aeruginosa* denitrification, accounts for the defect of PAO1 Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains during anaerobic growth and survival, particularly in biofilms.

3.1.3 Anaerobic colony biofilm growth of stringent response deficient *Pseudomonas aeruginosa* strains is restored by deletion of *nirS*

To determine the molecular mechanism which impairs anaerobic growth of PAO1 Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains (3.1.1 and 3.1.2), a contribution of nitric oxide to the observed phenotype was investigated. Accumulation of nitric oxide is abolished in a Δ*nirS* mutant strain (Barraud *et al.*, 2006), which does not form the nitrite reductase mediating the reduction of nitrite to nitric oxide during denitrification. To determine if nitric oxide causes the anaerobic growth defect of Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains, *nirS* was deleted in these strains and anaerobic colony biofilm growth of the respective mutant strains investigated (Fig. 16, 17 and 18).

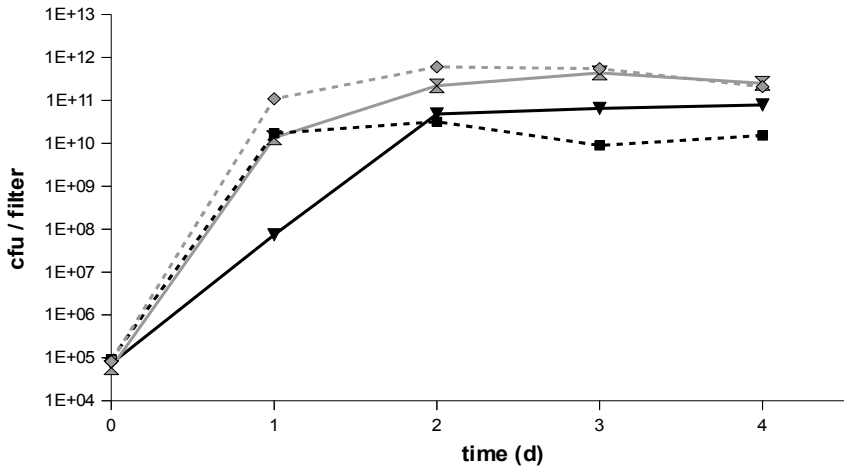

Fig. 16: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*nirS*, Δ*relA*Δ*spoT* and Δ*relA*Δ*spoT*Δ*nirS.* Colony biofilms of wild type (gray dashed line, diamonds), Δ*nirS* (gray solid line, hourglasses), Δ*relA*Δ*spoT* (black dashed line, squares) and Δ*relA*Δ*spoT*Δ*nirS* (black solid line, triangles) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), values represent the average of two independent experiments.

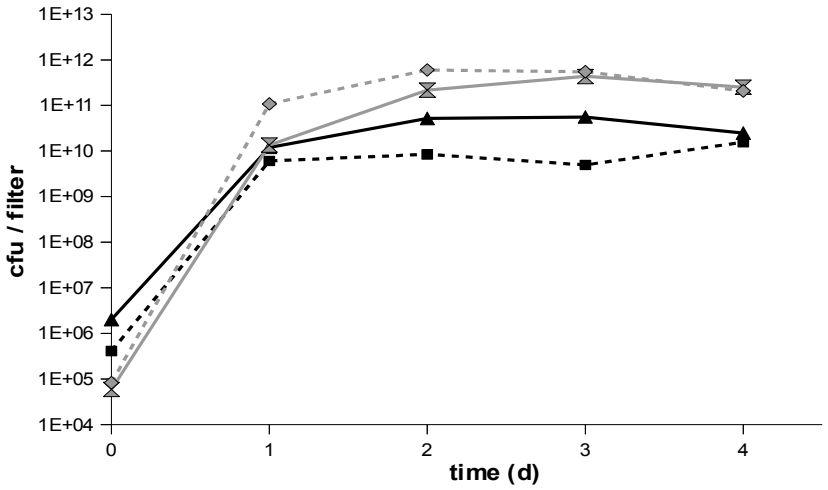

Fig. 17: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*nirS,* Δ*dksA* and Δ*dksA*Δ*nirS*. Colony biofilms of wild type (gray dashed line, diamonds), Δ*nirS* (gray solid line, hourglasses), Δ*dksA* (black dashed line, squares) and Δ*dksA*Δ*nirS* (black solid line, triangles) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), values represent the average of two independent experiments.

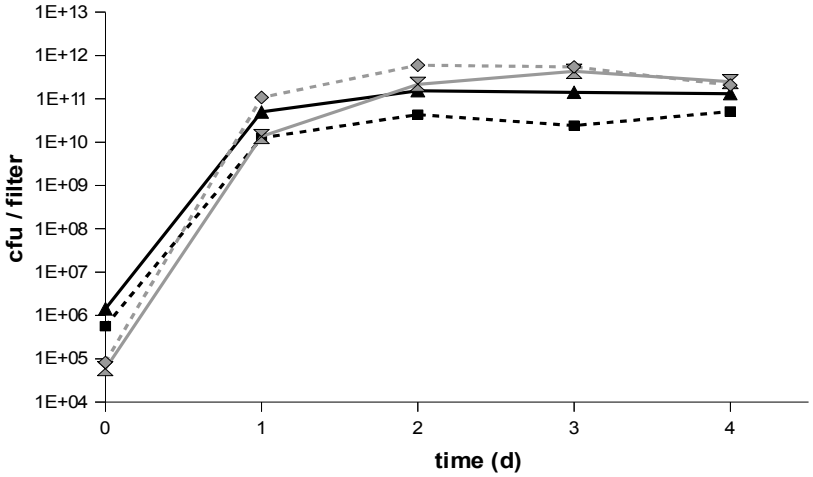

Fig. 18: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1 and mutant strains Δ*nirS*, Δ*relA*Δ*spoT* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA*Δ*nirS.* Colony biofilms of wild type (gray dashed line, diamonds), Δ*nirS* (gray solid line, hourglasses), Δ*relA*Δ*spoT*Δ*dksA* (black dashed line, squares) and Δ*relA*Δ*spoT*Δ*dksA*Δ*nirS* (black solid line, triangles) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), values represent the average of two independent experiments.

As seen in Fig. 16, deletion of *nirS* in PAO1 wild type results in slight delay of anaerobic colony biofilm growth. After one day of incubation, the wild type has reached approximately 1 x 10^{11} cfu/filter, whereas a Δ*nirS* mutant strain displays a ten-fold lower number of colony forming units. However, over the course of three days the Δ*nirS* mutant

strain reaches wild type levels and from this time point, both strains behave similarly.

Deletion of *nirS* causes different effects when introduced in Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* backgrounds. As seen in Fig. 16, a Δ*relA*Δ*spoT*Δ*nirS* mutant strain shows severely prolonged anaerobic colony biofilm growth, with approximately 1 x 10^8 cfu/filter detectable after one day of incubation, representing a 100-fold lower cell count than those observed for Δ*relA*Δ*spoT.* However, this delay is compensated after two days of biofilm growth after which Δ*relA*Δ*spoT*Δ*nirS* and Δ*relA*Δ*spoT* mutant strains show similar numbers of colony forming units. More importantly, during subsequent incubation the number of colony forming units are 10-fold increased in Δ*relA*Δ*spoT*Δ*nirS* compared to Δ*relA*Δ*spoT* mutant strain.

As seen in Fig. 17 and 18, deletion of *nirS* has similar positive effects on anaerobic colony biofilm growth of Δ*dksA* as well as Δ*relA*Δ*spoT*Δ*dksA* mutant strains. After three days of incubation, the number of colony forming units observed for Δ*dksA*Δ*nirS* mutant strain is 10-fold higher compared to Δ*dksA* mutant strain, as well as for Δ*relA*Δ*spoT*Δ*dksA*Δ*nirS* compared to Δ*relA*Δ*spoT*Δ*dksA* mutant strain. However, neither Δ*dksA*Δ*nirS* nor Δ*relA*Δ*spoT*Δ*dksA* exhibit prolonged growth rates within the first two days, as observed for the Δ*relA*Δ*spoT* mutant strain.

These results suggest part of the anaerobic colony biofilm growth defect of *P. aeruginosa* mutant strains Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* can be compensated by inhibition of nitric oxide production via denitrification. Interestingly, different effects upon introduction of a *nirS* deletion in Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains is observed during planktonic anaerobic growth and survival (data not shown). In agreement with results obtained during anaerobic colony biofilm growth, almost similar survival rates of Δ*relA*Δ*spoT*Δ*nirS* and Δ*nirS* mutant strains were observed. However, neither Δ*dksA* or Δ*relA*Δ*spoT*Δ*dksA* mutant strain displayed increased anaerobic survival in planktonic cultures. These results implicate DksA might play a different role during anaerobic planktonic and colony biofilm growth.

In *P. aeruginosa* the level of nitric oxide generated and consumed via denitrification is determined by activities of dissimilatory nitrite reductase NirS and nitric oxide reductase NorBC. Therefore, a tight regulation of *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* gene expression is essential for cellular survival and maintenance of biofilm growth.

Although the aerobic response to nitrosative stress is well investigated, only a few genes were shown to mediate nitric oxide protection in the absence of oxygen. Gene products of *hmgA* and *hpd*, encoding homogentisate-1,2-dioxygenase and 4-hydroxyphenylpyruvate dioxygenase, respectively, were shown to play role in protection of a PAO1 Δ*norBC* mutant strain from nitric oxide mediated stress during anaerobic conditions (Yoon *et al*., 2007). The *P. aeruginosa* genome harbors more than 20 genes which encode potential

dioxygenases possibly participating in nitric oxide detoxification.

In order to determine why the absence of RelA and SpoT or DksA enhances nitric oxide mediated stress in *P. aeruginosa,* transcriptome profiles of PAO1 mutant strains Δ*relA*Δ*spoT,* Δ*dksA* and wild type were analyzed. These gene expression data also allows a better understanding of ppGpp- and DksA-mediated gene regulation during the *P. aeruginosa* stringent response.

3.1.4 Transcriptome analysis during the anaerobic *Pseudomonas aeruginosa* stringent response

3.1.4.1 Induction of the stringent response with SHX and NaOH

P. aeruginosa PAO1 strains unable to synthesize ppGpp or regulator DksA, have a severe defect during anaerobic stationary phase survival (3.1.1 and 3.1.2), which is at least partially caused by lethal effects of nitric oxide (3.1.3). Transcriptome analysis with mutant strains Δ*relA*Δ*spoT* and Δ*dksA* was carried out in order to determine if the absence of ppGpp or DksA increases nitric oxide mediated stress either by an elevated production or a defective protection. In addition, rendering Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains unable to produce nitric oxide did not completely restore anaerobic colony biofilm growth, suggesting the stringent response may participate in regulation of other factors contributing to regular biofilm growth. ppGpp- or DksA-regulated genes involved in biofilm growth might be identified via transcriptome analysis.

Since an appearance of suppressor mutants during anaerobic colony biofilm of PAO1 Δ*relA*Δ*spoT* was observed within two days of incubation (3.1.2), cells for transcriptome analysis were harvested from planktonically grown cultures in which the stringent response was triggered for short time periods of 15 minutes (experimental procedures carried out by Nelli Bös). For that purpose, cells from the mid-exponential growth phase were treated with either SHX or NaOH. The amino acid analog SHX induces a RelA-dependent stringent response by mimicking amino acid acid starvation (Tosa & Pizer, 1971), addition of 20 mM NaOH adjusts the culture to a pH of 9, inducing a SpoT-dependent stringent response although the exact mechanism is not understood (Boes *et al*., 2008). Both experiments, induction of the stringent response with SHX as well as NaOH, were carried out with mutant strains Δ*relA*Δ*spoT,* Δ*dksA* and wild type.

For transcriptome analysis, PAO1 Δ*relA*Δ*spoT*, Δ*dksA* and wild type were grown anaerobically to an OD_{578} of 0.5 and subsequently treated with either SHX or NaOH for 15 minutes (Fig. 19). Total RNA was prepared (2.6.11) and reverse transcribed, the resulting cDNA was fragmented, labeled and hybridized to *P. aeruginosa* Affymetrix

GeneChips (2.9.1).

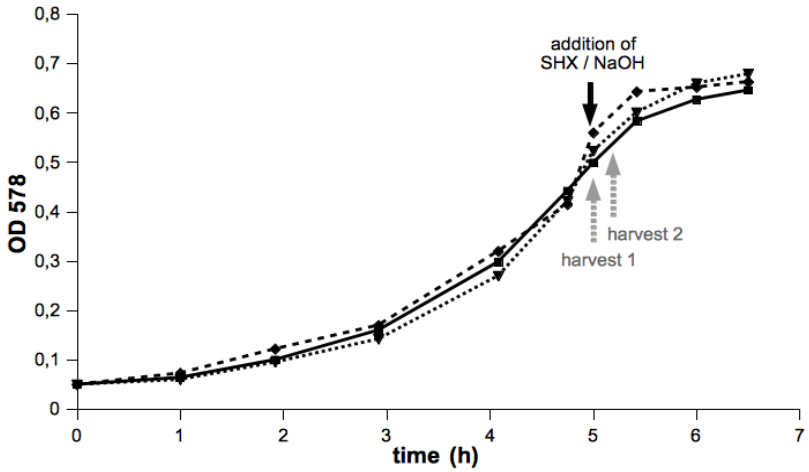

Fig. 19: Anaerobic planktonic growth of *Pseudomonas aeruginosa* PAO1 wild type and mutant strains Δ*relA*Δ*spoT* and Δ*dksA* and subsequent induction of the stringent response for transcriptome analysis. Cultures of wild type (solid line, squares), Δ*relA*Δ*spoT* (dashed line, diamonds) and Δ*dksA* (dotted line, triangles) were grown anaerobically in sealed serum flasks in LB supplemented with 50 mM KNO_3. At an OD_{578} of 0.5 the stringent response was induced by addition of either 3 mM SHX or 20 mM NaOH (black arrow). Prior to ("harvest 1") and 15 minutes after ("harvest 2") induction of the stringent response, cells were harvested and total RNA was prepared (2.6.11). RNA was reverse transcribed into cDNA, which was fragmented, labeled and hybridized to *P. aeruginosa* Affymetrix GeneChips (2.9.1). Experimental procedures were carried out by Nelli Bös.

Obtained raw microarray data was preprocessed, fold change expression values calculated (2.9.1) and transcriptome profiles were analyzed. Although the stringent response affects transcriptional profiles primarily in stationary phase, the absence of RelA and SpoT or DksA in the respective mutant strains also influences expression of several genes during exponential growth. In order to determine ppGpp- and DksA-dependent gene expression patterns only in response to simulated stringent response conditions, genotype-specific changes of gene expression prior to addition of SHX or NaOH were excluded from analysis. The same was true for ppGpp- and DksA-independent changes in gene regulation upon the addition of SHX or NaOH. To fulfill these criteria, transcriptome data was analyzed according to the diagram depicted in Fig. 20.

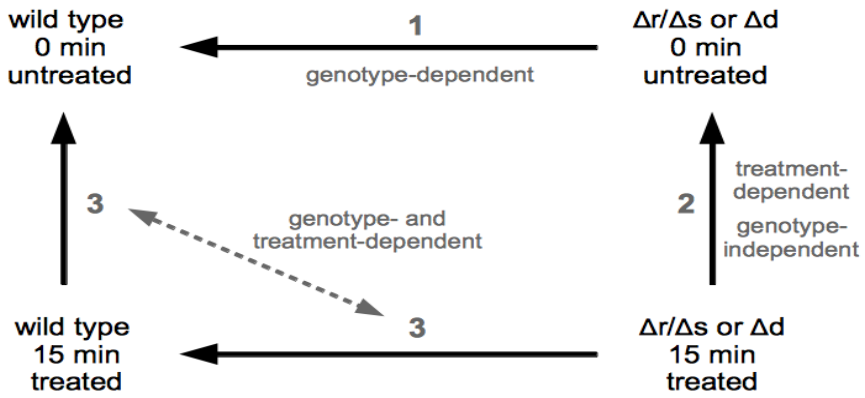

Fig. 20: Schematic overview of transcriptome data analysis for investigation of ppGpp- and DksA-dependent gene regulation in *Pseudomonas aeruginosa* PAO1. "Δr/Δs" refers to Δ*relA*Δ*spoT* mutant strain, "Δd" to Δ*dksA* mutant strain. "treated" refers to treatment with either SHX or NaOH and "untreated" to cultures prior to SHX- or NaOH treatment. (1) Genotype-specific gene regulation prior to treatment was excluded from analysis by comparing untreated mutant cultures to untreated wild type cultures. (2) Treatment-dependent but genotype-independent gene regulation was excluded from data analysis by discarding genes which were differentially regulated in treated mutant cultures compared to untreated mutant cultures. (3) Differentially regulated genes which were not excluded in (1) or (2) were identified by comparing treated wild type cultures to untreated wild type cultures. Identified genes should show a reciprocal regulation in treated wild type cells compared to treated mutant cells, all genes which failed to comply this criteria were discarded from further analysis.

Transcriptome data was analyzed by cross-examination of expression profiles as seen in Fig. 20. Genes regarded as truly ppGpp- or DksA-dependent required a reciprocal regulation under the following conditions: treated wild type cells compared to untreated wild type cells, and treated wild type cells compared to treated mutant cells. Differentially regulated genes of untreated wild type and mutant cells were compared to exclude any effects of Δ*relA*Δ*spoT* or Δ*dksA* backgrounds prior to SHX or NaOH treatment, respectively. Genes which were differentially regulated in treated mutant samples in respect to untreated mutant cells were also not further examined in this study, as they are regulated in a treatment-dependent but not ppGpp- or DksA-dependent manner. Exempted from this rule were genes which were regulated in treated mutant samples in respect to untreated mutant cells, but showed a significantly higher regulation in treated wild type cells compared to untreated wild type cells..

After cross-examination of transcriptome profiles, ppGpp- and DksA-dependent gene regulation in response to SHX or NaOH treatment was further characterized.

3.1.4.2 General ppGpp- and DksA-mediated transcriptional response to SHX and NaOH treatment

The amino acid analog SHX mimics amino acid acid starvation, which activates the ribosome-associated pppGpp synthetase RelA, resulting in the formation of the alarmone ppGpp. In addition to RelA-mediated gene regulation, it was also investigated how co-regulatory protein DksA affects gene expression upon SHX treatment of anaerobically grown *P. aeruginosa*.

After cross-examination of samples (Fig. 20), a total number of 295 genes were regarded as differentially regulated by ppGpp in response to SHX treatment. Of these genes, 94 were induced and 201 repressed by ppGpp. Appendix A lists all genes regulated by ppGpp upon SHX treatment of anaerobically grown cultures and their respective fold changes. Fig. 21 depicts a functional classification of genes differentially regulated by ppGpp in response to simulated amino acid starvation.

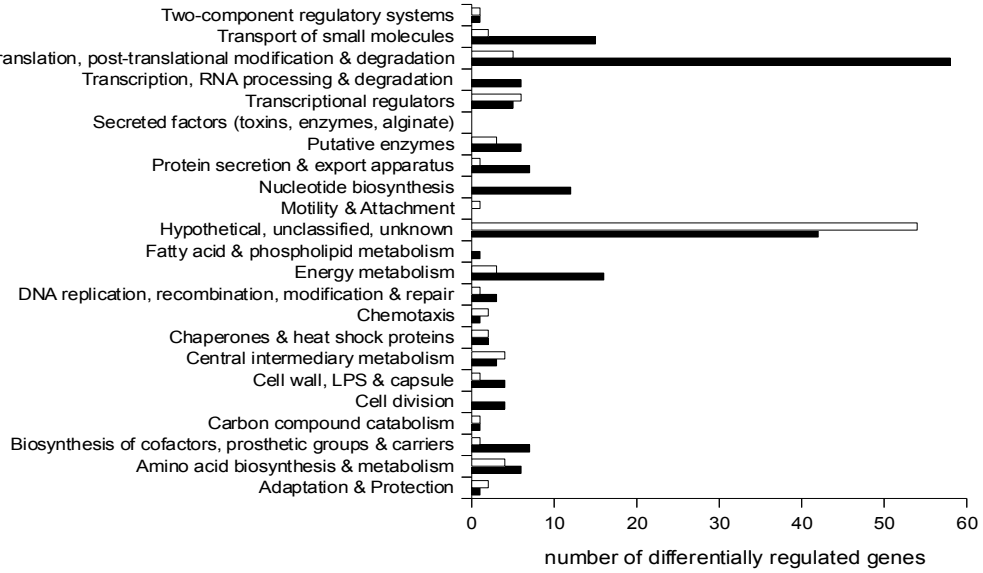

Fig. 21: Functional classification of genes differentially regulated by ppGpp after 15 minutes of SHX treatment of anaerobically grown *Pseudomonas aeruginosa* PAO1. Genes are classified according to "*Pseudomonas* Genome Database". White bars represent induced genes, black bars repressed genes.

After cross-examination of samples (Fig. 20), a total number of 364 genes were regarded as differentially regulated in a DksA-dependent manner in response to SHX treatment. Of these genes, 114 were induced and 250 repressed by DksA. Appendix B lists all genes regulated in a DksA-dependent manner upon SHX treatment of anaerobically grown cultures and their respective fold changes. Fig. 22 depicts a functional classification of genes differentially regulated by DksA in response to simulated amino acid starvation.

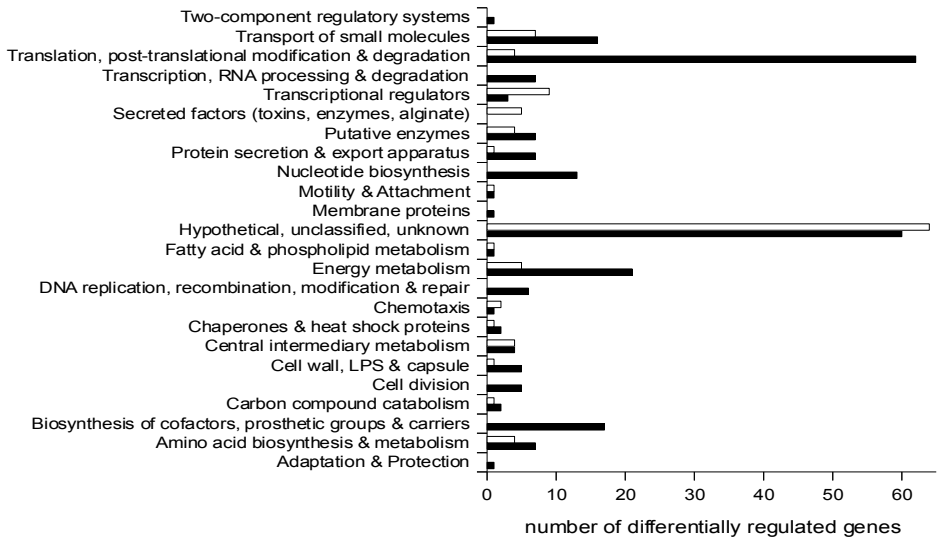

Fig. 22: Functional classification of genes differentially regulated by DksA after 15 minutes of SHX treatment of anaerobically grown *Pseudomonas aeruginosa* PAO1. Genes are classified according to "*Pseudomonas* Genome Database". White bars represent induced genes, black bars repressed genes.

Adjusting a *P. aeruginosa* culture to a pH of 9 with 20 mM NaOH induces ppGpp synthesis in a SpoT-dependent manner. In addition to the effect of SpoT on gene regulation, it was also investigated how DksA affects gene expression upon NaOH treatment of anaerobically grown *P. aeruginosa*.

After cross-examination of samples (Fig. 20), a total number of 204 genes were regarded as differentially regulated in a ppGpp-dependent manner in response to NaOH treatment. Of these genes, 77 were induced and 127 repressed by ppGpp. Appendix C lists all genes regulated by ppGpp upon NaOH treatment of anaerobically grown cultures and their respective fold changes. Fig. 23 depicts a functional classification of genes differentially regulated by ppGpp in response to NaOH treatment.

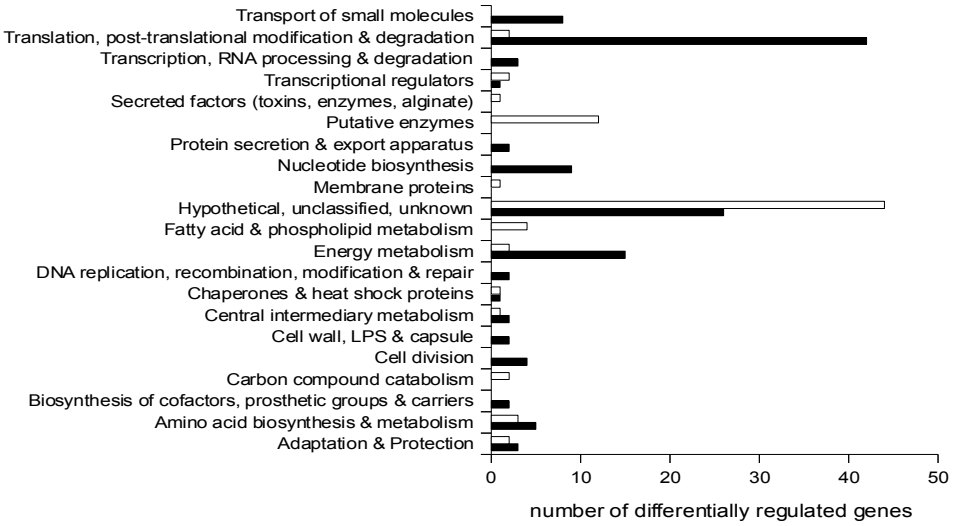

Fig. 23: Functional classification of genes differentially regulated by ppGpp after 15 minutes of NaOH treatment of anaerobically grown *Pseudomonas aeruginosa* PAO1. Genes are classified according to "*Pseudomonas* Genome Database". White bars represent induced genes, black bars repressed genes.

After cross-examination of samples (Fig. 20), a total number of 244 genes were regarded as differentially regulated in a DksA-dependent manner in response to NaOH treatment. Of these genes, 97 were induced and 147 repressed by DksA. Appendix D lists all genes regulated by DksA upon NaOH treatment of anaerobically grown cultures and their respective fold changes. Fig. 24 depicts a functional classification of genes differentially regulated by DksA in response to NaOH treatment.

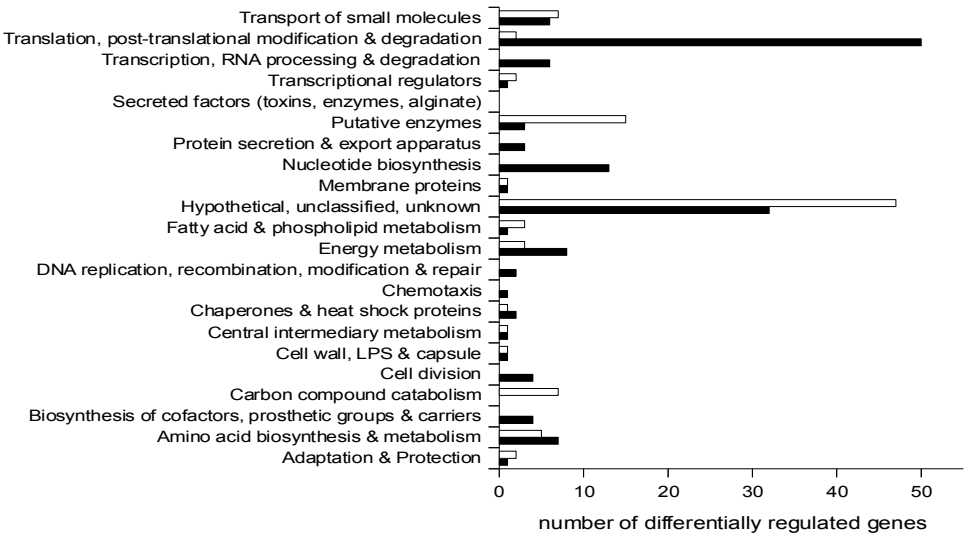

Fig. 24: Functional classification of genes differentially regulated by DksA after 15 minutes of NaOH treatment of anaerobically grown *Pseudomonas aeruginosa* PAO1. Genes are classified according to "*Pseudomonas* Genome Database". White bars represent induced genes, black bars repressed genes.

Activation of the stringent response upon starvation or other stress conditions requires a rapid growth arrest of the bacterial cell. Consequently, as shown in a number of bacteria, accumulation of ppGpp results in a downregulation of genes involved in cellular growth processes, i. e. translation, replication, energy metabolism or cell division (Magnusson *et al*., 2005). As seen in Fig. 21, 22, 23 and 24, induction of the stringent response with either SHX or NaOH in *P. aeruginosa* reveals a similar gene expression pattern, mediated by both ppGpp and DksA. A large number of genes involved in translation and ribosome biogenesis were repressed in a ppGpp-and DksA-dependent manner. A downregulation was also observed for genes encoding products which participate in energy metabolism, nucleotide biosynthesis and cell division, indicating a growth arrested state upon induction of the stringent response.

Expression of gene PA4853, encoding a protein with 80 % homology to the *E. coli* DNA-binding protein Fis (factor for inversion stimulation), was repressed in a ppGpp- and DksA dependent manner. Fis is a nucleoid-associated protein, which was shown to contribute to fundamental cellular processes in *E. coli* (Dorman, 2009). For instance, Fis is involved in replication initiation of the chromosome at *oriC* (Ryan *et al*., 2004) and was shown to be a cofactor for phage integration and excision from the chromosome (Esposito & Gerard, 2003). Fis also regulates transcription of a large number of bacterial genes (Grainger *et al.*, 2006; Kelly *et al.*, 2004), either by direct interaction with RNAP (McLeod *et al.*, 2002) or by modulation of DNA supercoiling structures of promoters (Auner *et al*., 2003). Transcription of promoters of genes encoding tRNA's and rRNA's is modulated by an interplay of Fis and other nucleoid-associated proteins (Hillebrand *et al*., 2005; Hirvonen *et al*., 2001). Protein levels of Fis peak during exponential growth and decrease at the entry to stationary phase (Keane & Dorman, 2003). The *E. coli fis* promoter was shown to be regulated via the stringent response (Mallik *et al.*, 2004), which is in agreement with a downregulation of PA4853 in a ppGpp- and DksA-dependent manner observed in this study.

Genes whose expression is positively affected by ppGpp at large contribute to bacterial survival, i. e. genes involved in general stress response or acquisition of nutrients such as amino acids (Magnusson *et al.*, 2005). For instance, ppGpp and DksA positively affect expression of the *himD* gene, encoding the β-subunit of the DNA-binding protein integration host factor (IHF). IHF has been shown to increase survival during acid- or oxidative stress and starvation, as well being required for virulence gene expression in various Gram-negative bacteria (Mangan *et al.*, 2006; Nyström, 1995). A ppGpp-dependent regulation of *E. coli* genes encoding α- and β-subunits of IHF was previously reported (Aviv *et al.*, 1994).

In conclusion, the ppGpp- and DksA-mediated transcriptional response of *P. aeruginosa* to

SHX or NaOH treatment features several characteristics implicated in the stringent response in other bacteria. However, analysis of transcriptome profiles also revealed ppGpp and DksA are involved in regulation of cellular processes not immediately connected to achieving a growth arrested state. Functional classification of genes differentially regulated by ppGpp and DksA also suggests that DksA is able to control expression of several genes independently of ppGpp. In order to distinguish ppGpp- and DksA-mediated gene expression, an overlay of the obtained transcriptome profiles was performed.

3.1.4.3 Interaction of ppGpp and DksA

Comparison of ppGpp- and DksA-mediated differential gene expression upon SHX or NaOH treatment of anaerobically grown *P. aeruginosa* cells, revealed an interesting regulatory network controlled by the stringent response.

In order to distinguish the influences of ppGpp and DksA on this regulatory network, it was first determined if transcription of *dksA* is affected by ppGpp or DksA in turn affects expression of *relA* and *spoT* required for ppGpp synthesis. As seen in Table 10, transcriptome analysis revealed that RelA, SpoT and DksA indeed influence each other's gene expression prior and upon induction of the stringent response.

Table 10: Differential expression of genes *relA*, *spoT* and *dksA* in response to SHX or NaOH treatment of anaerobically grown *Pseudomonas aeruginosa* PAO1. Details on experimental procedures and data analysis are described elsewhere (3.1.4.1). 'wt' indicates wild type, 'Δr/Δs' Δ*relA*Δ*spoT* mutant strain and 'Δd' Δ*dksA* mutant strain. Values depicted in brackets indicate fold change expression values lower than 2-fold, gray fields represent irrelevant queries.

compared condition	fold change expression of *dksA*	fold change expression of *relA*	fold change expression of *spoT*
Δr/Δs vs. wt	(1.03)		
Δd vs. wt		0.37	0.06
wt SHX vs. wt	0.37	(0.95)	(0.70)
Δr/Δs SHX vs. wt SHX	2.45		
Δd SHX vs. wt SHX		0.49	0.10
wt NaOH vs. wt	(0.75)	(0.94)	0.38
Δr/Δs NaOH vs. wt NaOH	(1.36)		
Δd NaOH vs. wt NaOH		0.39	0.16

Based on fold change expression values depicted in Table 10, several regulatory cascades were deduced. A 0.37-fold downregulation of *relA* expression and a 0.06-fold downregulation of *spoT* expression in Δ*dksA* mutant strain compared to wild type prior to

addition of SHX or NaOH, suggests a positive effect of DksA on *relA* and *spoT* transcription during exponential growth. Meanwhile, expression of *dksA* was not affected by the deletion of *relA* and *spoT* during exponential growth. The results indicate that DksA may be required for maintenance of high cellular levels of RelA and SpoT during exponential growth, which is essential for a rapid synthesis of ppGpp during stress or starvation conditions.

Gene expression profiles observed upon induction of the stringent response were obtained after 15 minutes of exposure to either SHX or NaOH. Although it is unlikely these changes affect the initial stringent response, they may reflect an adaptation to the respective stress condition over a longer period.

SHX treatment of wild type cells does not affect expression levels of *relA* and *spoT* in comparison to untreated wild type cells, whereas *relA* and *spoT* are downregulated (0.49-fold and 0.10-fold, respectively) in SHX-treated Δ*dksA* mutant strain compared to SHX-treated wild type. These findings suggest the positive effect of DksA on *relA* and *spoT* expression observed during exponential growth remains existent at the initial stages of the stringent response. However, expression of *dksA* itself is repressed (0.37-fold) in a RelA- and SpoT-dependent manner in response to SHX treatment. As a consequence, the positive effect DksA exerts on *relA* and *spoT* transcription might be, at least in part, diminished once ppGpp accumulates over time in response to SHX treatment. Gene expression patterns of *relA*, *spoT* and *dksA* observed during NaOH treatment resemble those observed during SHX treatment, albeit fold change expression values are less pronounced (Table 10).

These findings suggest that during exponential growth, DksA mediates high cellular levels of RelA and SpoT, which are required for a rapid synthesis of ppGpp upon an external stimulus. However, after initial adaptation to a prevalent stress condition, an excess of ppGpp in the cell is likely to be unfavorable, as it interferes with adaptation to the prevalent condition or even subsequent growth. While ppGpp levels are controlled on protein level, transcriptome profiles indicate a long term cellular adaptation via differential gene expression under stress conditions. The gene regulatory changes described in the previous paragraph indicate a model where both *dksA* and *relA/spoT* are inter-dependently downregulated for adaptation to a long term stress condition. However, additional experiments are necessary to confirm these findings.

In conclusion, the regulatory cascade deducted from transcriptome analysis allows adaptation of *P. aeruginosa* to a prevalent stress or starvation condition which is initially mediated by ppGpp but independent of the stringent response at later stages. Fig. 25 summarizes the proposed regulatory interplay of RelA, SpoT and DksA during exponential growth and upon induction of the stringent response with either SHX or NaOH.

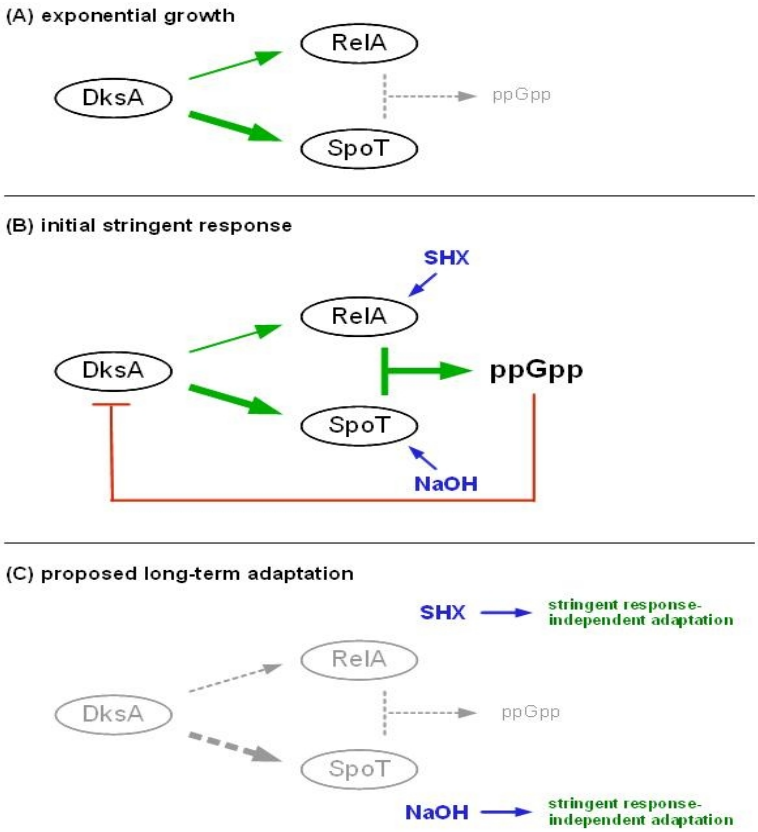

Fig. 25: Proposed inter-dependent regulatory network of RelA, SpoT and DksA which mediate the *Pseudomonas aeruginosa* stringent response. Deductions are made based on transcriptome analysis described described elsewhere (3.1.4.1). (A) During exponential growth DksA positively affects transcription of *relA* and *spoT*, resulting in high levels of proteins RelA and SpoT, which allows a rapid adaptation to stress or starvation conditions. During exponential growth, (p)ppGpp does not accumulate, since RelA and SpoT synthetases are not active. (B) After 15 minutes of SHX or NaOH treatment, activation RelA or SpoT resulted in an increased synthesis of (p)ppGpp. Elevated (p)ppGpp levels directly or indirectly mediate a decrease in *dksA* expression. (C) Proposed long-term adaptation to induction of the stringent response via SHX or NaOH. Decreased DksA protein levels diminish the positive effect of DksA on *relA* and spoT expression, resulting in low RelA and SpoT protein levels and consequently, low (p)ppGpp levels. This predicted long-term adaptation allows the cell to adjust to a prevalent stress condition via stringent response independent mechanisms.

It has been previously suggested that DksA acts as a downstream mediator of ppGpp-dependent gene expression in *E. coli,* although the exact mechanism of this interaction is uncertain (Brown *et al*., 2002). Transcriptome analysis described in this study suggests DksA, RelA and SpoT participate in a more complex regulatory cascade in *P. aeruginosa.* Additionally, several genes appear to be regulated by only ppGpp or DksA, implicating DksA is more than a cofactor required for the action of ppGpp. This notion is supported by results obtained for *E. coli*, in which overproduction of DksA compensated the loss of ppGpp in several cellular processes (Magnusson *et al.*, 2007). In some cases ppGpp and DksA even seem to have opposing roles, for instance in regulation of *E. coli* fimbriation (Aberg *et al.*,

2008). Transcriptome analysis in *E. coli* revealed that a number of genes encoding factors contributing to chemotaxis or flagellum biosynthesis were upregulated in a Δ*dksA* mutant strain but downregulated in a Δ*relA*Δ*spoT* mutant strain (Aberg *et al.*, 2009).
In order to further characterize differences and similarities of ppGpp- and DksA-mediated transcription, gene expression profiles during SHX and NaOH treatment of anaerobically grown *P. aeruginosa* were analyzed in detail.

3.1.4.4 Concurrence of ppGpp- and DksA-mediated gene regulation

In order to identify cellular processes controlled solely by ppGpp or DksA in *P. aeruginosa*, transcriptome profiles obtained during SHX or NaOH treatment of mutant strains Δ*relA*Δ*spoT*, Δ*dksA* and wild type were cross-examined (Fig. 26).

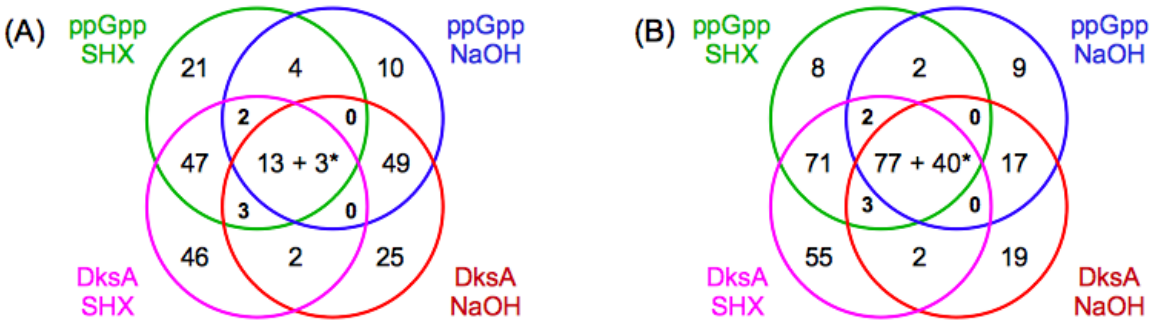

Fig. 26: Venn diagrams of genes differentially regulated by ppGpp and/or DksA in *Pseudomonas aeruginosa* PAO1 in response to SHX and/or NaOH treatment. Experimental procedures and data analysis of discussed transcriptome profiles are described elsewhere (3.1.4.1). Depicted are the number of genes (A) induced or (B) repressed by ppGpp and/or DksA during condition indicated. Note that genes which were found differentially regulated during three of four analyzed conditions (indicated by asterisk) are also regarded as part of the core regulon.

As seen in Fig. 26, ppGpp and/or DksA differentially regulate a total number of 530 genes upon induction of the stringent response with SHX or NaOH. Of these genes, 225 were induced and 305 repressed by ppGpp and/or DksA.
The largest number of genes was downregulated in a ppGpp- and DksA-dependent manner in response to both treatments. As expected this group includes many genes involved in active translation, nucleotide biosynthesis and cell division. ppGpp- and DksA-dependent gene regulation solely upon SHX treatment reveals a repression of similar classes of genes and also features several genes encoding small transporters, which are for instance involved in amino acid or C4-dicarboxylate transport. Meanwhile, in response to NaOH treatment a lower number of genes is regulated by both ppGpp and DksA, also covering genes involved in translation and energy metabolism. Very few genes are

regulated by ppGpp in response to both treatments, which is also true for DksA.

In contrast to the regulatory pattern observed for repressed genes, among the upregulated genes a significantly lower number was induced by both ppGpp and DksA in response to both stress conditions. Although genes of this category encode almost exclusively hypothetical or conserved hypothetical proteins, expression of the IHF-encoding *himD* gene is also positively affected during all conditions, as described before (3.1.4.2). A larger overlap was observed with regard to treatment-specific gene regulation mediated by both ppGpp and DksA, also consisting mainly of genes with unknown functions, with a few exemptions mentioned below. As observed for repressed genes, only a small number of genes is upregulated by either ppGpp or DksA in response to both conditions.

To gain a better understanding of the specific stringent response mediated adaptation of *P. aeruginosa* to SHX or NaOH, a detailed analysis of the genes regulated solely by ppGpp or DksA is of interest. Surprisingly, DksA induces a larger number of genes than ppGpp during the tested conditions, further demonstrating its importance during the *P. aeruginosa* stringent response.

Transcription of the *rpoS* gene, encoding the stationary phase specific σ^S factor (Potvin *et al*., 2007) is positively affected by ppGpp and DksA upon NaOH treatment. A regulation of *rpoS* expression by the stringent response is in agreement with previous reports. For instance, high ppGpp levels resulted in an increased *rpoS* transcription in *E. coli* (Gentry *et al.*, 1993) and a *P. aeruginosa* Δ*relA* mutant strain, which synthesizes less ppGpp than the wild type, had reduced although not abolished RpoS protein levels (Erickson *et al*., 2004). A *P. aeruginosa* Δ*rpoS* mutant strain was shown to be less resistant to high temperatures, hydrogen peroxide, low pH, hyperosmolarity and ethanol during stationary phase (Jorgensen *et al.*, 1999; Cochran *et al.*, 2000). Additionally, *P. aeruginosa* RpoS modulates expression of *rhlR* and *lasR*, encoding the transcriptional regulators of the AHL-dependent quorum sensing network (Latifi *et al*., 1996; Schuster *et al.*, 2004), implicating the stringent response may be important for *P. aeruginosa* quorum sensing.

The *algQ* gene, encoding one of two transcriptional regulators of alginate biosynthetic genes in *P. aeruginosa* (Deretic & Konyeecsni, 1989), was induced by ppGpp and DksA upon SHX treatment. Besides alginate biosynthesis, AlgQ participates in regulation of a number of cellular processes in *P. aeruginosa*, including biosynthesis of iron-chelating pyoverdine (Ambrosi *et al.*, 2005) and negative modulation of quorum sensing regulatory genes *lasR* and *rhlR* (Ledgham *et al.*, 2003). Interestingly, stationary phase survival of a *P. aeruginosa* Δ*algQ* mutant strain was greatly diminished due to low intracellular levels of polyphosphate, produced by a nucleoside diphosphate kinase expressed in an AlgQ-dependent manner (Kim *et al.*, 1998). It has been suggested that AlgQ positively

modulates ppGpp levels, which were drastically reduced in a *P. aeruginosa* Δ*algQ* mutant strain under low phosphate conditions (Kim *et al.*, 1998). Transcriptome analysis carried out in this study implicates ppGpp, in concert with DksA, actually induces *algQ* expression, suggesting the protein positively affects its own expression in an autoregulatory mechanism.

An increased intracellular concentration of the σ^{70} factor was also shown to mimic a Δ*algQ* phenotype, suggesting that AlgQ may act as an anti-σ^{70} factor (Ambrosi *et al.*, 2005). Although ppGpp and DksA actively repress σ^{70} encoding *rpoD* during SHX treatment, an increased *algQ* transcription upon the activation of the stringent response may further diminish σ^{70}-mediated transcription.

Several Gram-negative bacteria have been shown to propagate on semisolid surfaces via swarming motility, a cellular process mediated by flagella, type IV pili and rhamnolipids in *P. aeruginosa* (Köhler *et al.*, 2000), which is associated with changes in cellular morphology. *P. aeruginosa* swarmer cells were shown to be elongated and possess two polar flagella instead of one (Köhler *et al.*, 2000). However, the regulatory mechanism underlying *P. aeruginosa* swarming motility is at large unknown and may depend on the prevalent environmental condition. For instance, quorum sensing was shown to positively regulate swarming motility when *P. aeruginosa* was grown on succinate, but no regulatory influence was observed when either glucose or glutamate served as carbon source (Shrout *et al.*, 2006).

Several genes positively regulated by ppGpp or DksA were previously shown to contribute to *P. aeruginosa* swarming. ppGpp controls induction of transcriptional regulator encoding gene PA2897 upon SHX treatment, which was previously found upregulated during swarming conditions (Overhage *et al.*, 2008). Expression of the *rnk* gene, encoding a nucleoside diphosphate kinase regulator, was induced during swarming compared to swimming conditions in PA14 (Overhage *et al.*, 2008) and upregulated in a ppGpp-dependent manner upon SHX treatment in this study. Gene *pilB*, involved in biogenesis of type IV pili (Nunn *et al.*, 1990) and required for *P. aeruginosa* swarming, was induced by both ppGpp and DksA in response to SHX treatment.

Although the stringent response upregulates a number of genes contributing to *P. aeruginosa* swarming motility, several genes previously reported to be involved in swarming motility were repressed by ppGpp. For instance, ppGpp exerted a negative effect on the expression of *nosR* upon SHX treatment. Besides its participation in regulation of the *P. aeruginosa* denitrification chain (Arai *et al.*, 2003), NosR also was reported to contribute to swarming motility, as a PA14 Δ*nosR* mutant strain was severely affected in its ability to swarm (Yeung *et al.*, 2009). ppGpp and DksA also exert a negative control on the expression of *fis* encoding the global transcriptional regulator Fis. A PA14

Δ*fis* mutant strain was severely affected in its swarming ability during aerobic conditions (Yeung *et al*., 2009). Lastly, SHX treatment results in a ppGpp-dependent induction of the cold shock protein encoding *cspD*, whereas a PA14 Δ*cspD* mutant strain exhibits increased swarming ability (Yeung *et al.*, 2009).

In conclusion, these obtained results suggest ppGpp and DksA may affect *P. aeruginosa* swarming, but a detailed understanding of this process requires further analysis. If the stringent response indeed contributes to swarming motility, its role in *P. aeruginosa* biofilm growth is of interest with regard to this study. A current model suggests that only the presence of motile and non-motile cells leads to the formation and maturation of complete biofilm structures (Klausen *et al*., 2003).

DksA has been previously described to contribute to *P. aeruginosa* virulence via posttranscriptional regulation of several important virulence factors (Jude *et al*., 2003). In addition to these results and agreement with a role of DksA in regulation of virulence factors, SHX treatment resulted in a DksA-dependent upregulation of genes *aprDEFX* encoding an alkaline protease (Duong *et al.*, 1992), *hcpA* encoding a major exported protein (Lesic *et al.*, 2009) and *phzB1* involved in pyocyanine biosynthesis (Mavrodi *et al*., 2001). In addition, upon NaOH treatment ppGpp positively affects transcription of the *plcB* gene, encoding phospholipase C which also contributes to *P. aeruginosa* virulence. Interestingly, PlcB was shown be required for PAO1 twitching motility towards phosphatidylethanolamine, which is a substrate for PlcB (Barker *et al.*, 2004), suggesting another link between the stringent response and *P. aeruginosa* motility.

Transcriptome analysis carried out in this study also revealed that ppGpp and DksA induce several genes involved in *P. aeruginosa* type VI secretion. In the genome of *P. aeruginosa*, factors contributing to type VI secretion are organized in three pathogenicity islands, called HSI (Hcp-secretion island) clusters 1 to 3 (Mougous *et al*., 2006). Type VI secretion contributes to *P. aeruginosa* pathogenesis, as several single gene deletions within the HSI gene clusters attenuate virulence in chronic infection models (Potvin *et al.*, 2003). Proteins Hcp and VgrG, which are secreted via the type VI secretion system, are thought to mediate pathogenesis, although it is uncertain whether they are effector proteins or compounds of the type VI secretion system (Cascales, 2008). In this study, expression of several genes from HSI-1 and HSI-2 clusters was induced by both ppGpp and DksA in response to SHX treatment, suggesting an important role of the stringent response in *P. aeruginosa* virulence.

ppGpp and in particular DksA positively regulate a number of genes involved in fatty acid and carbon compound catabolism. For instance, the operon PA0744 – PA0747, likely participating in β-oxidation of fatty acids (Miller *et al.*, 2008), is upregulated in a SpoT- and DksA-dependent manner.

DksA also positively affects expression of gene *gpuR* upon SHX treatment. Transcriptional regulator GpuR induces the *P. aeruginosa gpuPA* operon encoding for enzymes of 3-guanidinopropionate catabolism, which is converted to acetyl-CoA via β-alanine (Nakada & Itoh, 2005). Expression of *liuR* (alternately named *gnyR*), encoding a transcriptional regulator which mediates the expression of the *P. aeruginosa liuABCDE* (*gnyDBHAL*) operon, is also positively regulated by DksA. Gene products of the *liuABCDE* operon participate in the leucine/isovalerate utilization (Liu) pathway (Förster-Fromme *et al.*, 2006). 3-methyl-crotonyl-CoA, which is generated from leucine or isovalerate by LiuA, is also formed via the acylic terpene utilization (Atu) pathway. Neither *luiA* nor genes encoding for the Atu pathway were induced in a DksA-dependent manner upon SHX treatment of anaerobically grown *P. aeruginosa*. However, expression of *liuCDE,* whose gene products mediate the three step generation of acetoacetate and acetyl-CoA from 3-methyl-crotonyl-CoA, was upregulated by DksA.

DksA also induces expression of genes *dhcAB*, encoding a dehydrocarnitine-CoA transferase involved in the catabolism of carnitine to glycine (Wargo & Hogan, 2009). In some bacteria, including *P. aeruginosa*, carnitine can serve as the sole source of carbon and nitrogen (Aurich & Lorenz, 1959). Lastly, DksA positively controls transcription of genes *pcaCD* in response NaOH treatment, whose products participate in the β-ketoadipate pathway (Kemp & Hegemann, 1968) by which aromatic compounds such as benzoate, coumarate or ferulate are degraded to tricarboxylic acid cycle (TCA) intermediates.

In conclusion, DksA affects several pathways contributing to the formation of acetyl-CoA which serves as a precursor for biosynthetic pathways, demonstrating an importance of the stringent response for the activation of metabolic pathways during nutrient limitation or other stresses.

As seen in this chapter ppGpp- and DksA-mediated gene regulation upon SHX or NaOH treatment in *P. aeruginosa* affects cellular functions other than mediating growth arrest by inhibition of translation, energy metabolism or cell division. These include regulation of genes whose products play a role in swarming motility, alginate biosynthesis, biofilm formation and pathogenesis but also the activation of metabolic pathways. Fig. 27 depicts a proposed regulatory network activated upon induction of the stringent response.

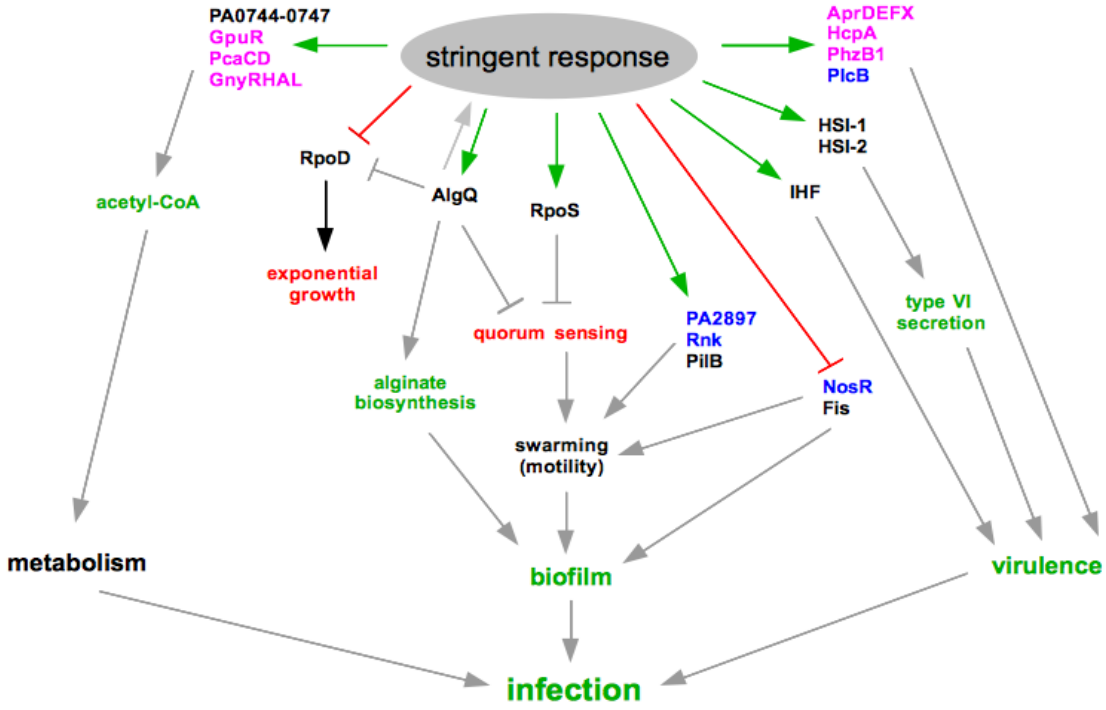

Fig.27: Proposed regulatory network of ppGpp- and DksA-dependent gene expression after 15 minutes of SHX and/or NaOH treatment of anaerobically grown *Pseudomonas aeruginosa* PAO1. Deductions were made based on transcription analysis described in this and preceding chapters. Gene products depicted black were found regulated by both ppGpp and DksA, gene products depicted in blue only by ppGpp and gene products depicted in magenta only by DksA. Green lines represent a positive regulation by the stringent response, red lines a negative regulation by the stringent response and gray lines regulatory connections indicated in literature. Cellular processes depicted in green are proposed to be positively affected by the stringent response, cellular processes depicted in red are proposed to be negatively affected by the stringent response. For details please refer to text.

3.1.4.5 ppGpp and DksA-dependent expression of genes involved in denitrification and nitric oxide protection

Severe anaerobic growth defects of *P. aeruginosa* PAO1 mutant strains Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* (3.1.1 and 3.1.2) might arise partly due to nitric oxide related damage (3.1.3). Transcriptome analysis was carried out to verify if the absence of RelA and SpoT or DksA deregulates genes involved in either denitrification or protection against nitrosative stress (3.1.4). To consider effects arising prior to SHX or NaOH treatment due to genotypes of Δ*dksA* and Δ*relA*Δ*spoT* mutant strains, expression profiles of treated mutant cultures compared to treated wild type cultures were analyzed. Table 11 lists all genes whose products are directly involved in denitrification and differentially regulated by ppGpp or DksA in response to SHX or NaOH treatment.

Table 11: Differential expression of genes involved in *Pseudomonas aeruginosa* PAO1 denitrification chain after SHX or NaOH treatment in Δ*relA*Δ*spoT*, Δ*dksA* and wild type under anaerobic denitrifying conditions. Experimental procedures and data analysis are described elsewhere (3.1.4.1). None of the enlisted genes was differentially expressed in Δ*relA*Δ*spoT* or Δ*dksA* prior to treatment. For detailed information on regulation and function of listed genes, see Appendix A, B, C and D. 'wt' describes wild type, 'Δr/Δs' Δ*relA*Δ*spoT* mutant strain and 'Δd' Δ*dksA* mutant strain. For the sake of clarity fold change expression values below two are not included in the table ('–').

PA number	gene name	FC Δr/Δs SHX vs. wt SHX	FC Δd SHX vs. wt SHX	FC Δr/Δs NaOH vs. wt NAOH	FC Δd NaOH vs. wt NAOH
PA3872	*narI*	–	–	–	0.40
PA3873	*narJ*	–	–	–	0.36
PA3873	*narH*	–	–	–	0.38
PA0509	*nirN*	–	0.45	–	0.39
PA0510	*nirE*	–	0.40	–	0.34
PA0511	*nirJ*	–	0.36	–	0.38
PA0512	*nirH*	–	0.41	–	0.44
PA0513	*nirG*	–	0.22	–	0.35
PA0514	*nirL*	–	0.36	–	0.37
PA0515	*nirD*	–	0.44	–	0.48
PA0516	*nirF*	–	0.40	–	0.48
PA0517	*nirC*	–	0.44	–	0.47
PA0520	*nirQ*	0.49	–	0.39	0.38
PA0521	*nirO*	–	–	0.33	0.28
PA0523	*norB*	–	–	–	–
PA0524	*norC*	–	–	–	–
PA0525	*norD*	–	0.37	–	–
PA3391	*nosR*	2.01	–	–	–

As seen in Table 11, expression of the majority of genes involved in denitrification is not severely affected in Δ*relA*Δ*spoT* mutant strain compared to wild type after either SHX or NaOH treatment. However, expression of *nirQ* was slightly reduced compared to wild type in response to both treatments. A larger number of genes whose products participate in denitrification were differentially regulated in Δ*dksA* mutant strain upon SHX or NaOH treatment. In response to both treatments various genes of the *nirSMCFDLGHJEN* operon, encoding the *P. aeruginosa* dissimilatory nitrite reductase (Zumft, 1997), were downregulated in treated Δ*dksA* mutant strain compared to treated wild type. NaOH treatment additionally resulted in repressive effects on transcription of genes *narIJH*, encoding catalytic or assembling compounds of the dissimilatory nitrate reductase (Zumft, 1997), in Δ*dksA* mutant strain.

Expression of genes encoding the NorBC nitric oxide reductase was not differentially regulated in either Δ*relA* Δ*spoT* or Δ*dksA* mutant strains, dissenting the hypothesis that

accumulation of nitric oxide mediates growth defects of anaerobic colony biofilms of Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains (3.1.3).

In a next step it was investigated if genes contributing to protection from nitric oxide related stress were regulated by ppGpp or DksA. *hpd* encodes a 4-hydroxyphenylpyruvate dioxygenase, which was shown to rapidly bind nitric oxide via its Fe^{2+} center and which protects a *P. aeruginosa* Δ*norB*C mutant strain from nitric oxide mediated killing (Yoon *et al.*, 2007). Expression of *hpd* was moderately (2.5-fold) induced by both ppGpp and DksA. Consequently, a decreased *hpd* transcription in PAO1 Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains might result in an increased nitric oxide stress. Expression of *hmgA*, encoding a second dioxygenase with the ability to scavenge nitric oxide (Yoon *et al.*, 2007), was not differentially regulated in a ppGpp- or DksA-dependent manner. However, transcription of gene PA1999 encoding an acetyl-CoA acetyltransferase was upregulated (11.5-fold) upon NaOH treatment in a DksA-dependent manner. Protein levels of PA1999 were also increased in an anaerobically grown stationary phase *P. aeruginosa* Δ*norB*C mutant strain compared to wild type (Yoon *et al.*, 2007).

In conclusion, although several genes of the *narK₁K₂GHIJLX* and in particular the *nirSMCFDLGHJEN* operon are differentially regulated by ppGpp and DksA upon induction of the stringent response, the observed regulatory pattern does not indicate an accumulation of nitric oxide via denitrification in the Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains. However, two gene whose products likely contribute to protective measures against nitric oxide related stress were downregulated in Δ*relA*Δ*spoT* and Δ*dksA* mutant strains compared to wild type.

3.1.4.6 ppGpp- and DksA-regulated genes with potential roles in *Pseudomonas aeruginosa* biofilm growth

It was shown that nitric oxide might contribute to the anaerobic growth defect of PAO1 Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains (3.1.3). However, a deletion of *nirS* did not completely restore wild type anaerobic colony biofilm growth of these mutant strains, suggesting other factors regulated by the stringent response are also important for anaerobic colony biofilm growth of *P. aeruginosa*. Transcriptome analysis carried out to identify ppGpp- and DksA-dependent genes (3.1.4.1) were further examined to identify factors which possibly contribute to anaerobic colony biofilm growth deficiencies of Δ*relA*Δ*spoT* and Δ*dksA*. Indeed, several genes which have been previously implicated to be involved in biofilm growth were deregulated in cells lacking RelA and SpoT or DksA.

Expression of *himD* was induced in PAO1 wild type cells upon SHX treatment (4.0-fold) as well as NaOH treatment (2.4-fold) by both ppGpp and DksA. The *P. aeruginosa himD*

gene encodes the β-subunit of the DNA binding protein integration host factor (IHF). IHF is a dimeric, DNA-binding protein which was shown to play a role in bacterial stress response, such as acid- or oxidative stress, survival during starvation conditions and virulence gene expression in various Gram-negative bacteria (Mangan *et al.*, 2006; Nyström, 1995). Also, IHF has been previously reported to enhance *E. coli* biofilm formation (García-Contreras *et al.*, 2008).

Genes PA1323 and PA1324 were highly upregulated (24.6-fold and 22.3-fold, respectively) in a ppGpp- as well as DksA-dependent manner upon NaOH treatment. Based on structural analysis the respective gene products were postulated to mediate transport of polysaccharide associated with the peptidoglycan matrix during biofilm formation (Mercier *et al.*, 2009). Additionally, expression of PA1323 and PA1324 was induced during both microaerobic and anaerobic growth of *P. aeruginosa* PAO1 (Alvarez-Ortega & Harwood, 2007).

Genes PA3729 to PA3732 were moderately induced (2.0- to 3.1-fold) in SHX-treated wild type cells by both DksA and ppGpp. The operon PA3729 to PA3732 was recently renamed to biofilm-associated cluster (*bac*), as a PAO1 PA3731::IS*phoA*/hah transposon insertion mutant displayed severely impaired biofilm growth and was also attenuated in its virulence in a mouse lung infection model (Macé *et al*., 2008).

In order to investigate if any of these genes is essential for functional anaerobic colony biofilm growth, a PAO1 Δ*himD* mutant strain as well as PA14 PA1323::MrT7, PA1324::MrT7, PA3729::MrT7, PA3730::MrT7, PA3731::MrT7 and PA3732::MrT7 transposon insertion mutant strains (Liberati *et al.*, 2006) were characterized. However, none of the mutant strains was affected in its ability to form colony biofilms during anaerobic conditions (data not shown). These result suggest that differential regulation of one of these genes by ppGpp or DksA does not contribute to impaired anaerobic biofilm colony growth of Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains. However, it is possible that single gene deletions do not reflect the situation which mediates the observed phenotype of Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains.

Gene *rpoS* encodes the stationary phase and stress responsive σ-factor σ^S which was previously reported to be required for maintenance of *P. aeruginosa* biofilm architecture (Heydorn *et al.*, 2002). Deletion of *rpoS* was shown to lead to the formation of significantly thicker biofilms in a flow-through biofilm models (Whiteley *et al.*, 2001). Triggering the stringent response with NaOH resulted in a 2.6-fold induction of *rpoS* expression by both ppGpp and DksA. To investigate if a deregulation of *rpoS* in mutant strains Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* affects anaerobic colony biofilm growth, PAO1 Δ*rpoS* mutant strain was screened in the biofilm model used in this study (Fig. 28).

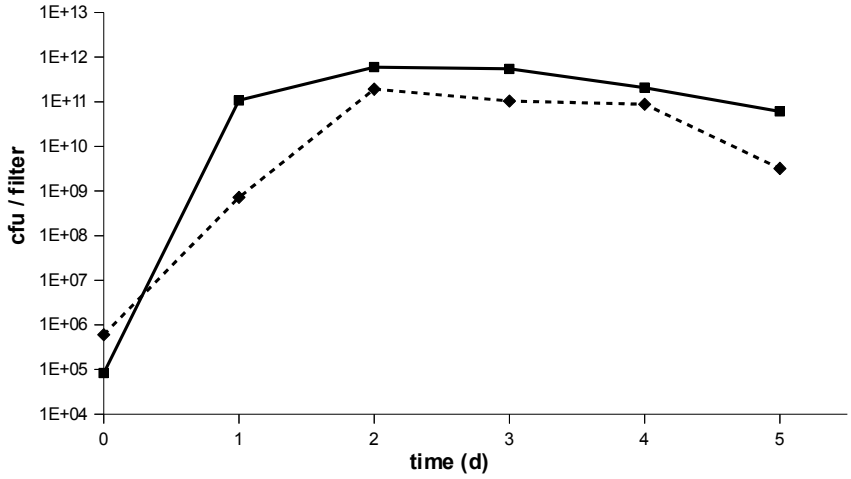

Fig. 28: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1 and mutant strain Δ*rpoS*. Colony biofilms of wild type (solid line, squares) and Δ*rpoS* (dashed line, diamonds) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), values represent the average of two independent experiments.

As seen in Fig. 28, deletion of *rpoS* severely attenuates anaerobic colony biofilm growth of *P. aeruginosa*. After one day of incubation approximately 5 x 10^8 cfu/filter were detected for Δ*rpoS* mutant strain, compared to approximately 1 x 10^{11} cfu/filter detected for PAO1 wild type. However, this delay is at large compensated after two days of incubation. Over the course of five days colony biofilms the Δ*rpoS* mutant strain displays an average 5-fold reduced cell count compared to wild type. Biofilms formed by the PAO1 Δ*rpoS* mutant strain used in this study were reported to form significantly thicker biofilms than the corresponding wild type (Whiteley *et al.*, 2001; Heydorn *et al.*, 2002). However, these results were obtained for biofilms grown in flow chambers which may not be comparable to the colony biofilm model applied in this study. The 5-fold reduced number of colony forming units observed for Δ*rpoS* mutant strain suggests a deregulation of *rpoS* expression might contribute to the impaired anaerobic colony biofilm growth defect of Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutant strains.

The *pfpI* gene was strongly induced in wild type cultures treated with NaOH by both ppGpp and DksA (10.8-fold). Recently, it was shown that both PAO1 Δ*pfpI* and PA14 Δ*pfpI* mutant strains are affected in their ability to form biofilms as well as more prone to H_2O_2 induced mutations (Rodríguez-Rojas & Blázquez, 2009). To investigate if a deregulation of *pfpI* in Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spo*Δ*dksA* mutant strains results in a defect during anaerobic colony biofilm growth, PAO1 *pfpI*::Is*lacZ*/hah and *pfpI*::Is*phoA*/hah transposon insertion mutant strains (Jacobs *et al.*, 2003) were screened in the biofilm model applied in this study (Fig. 29).

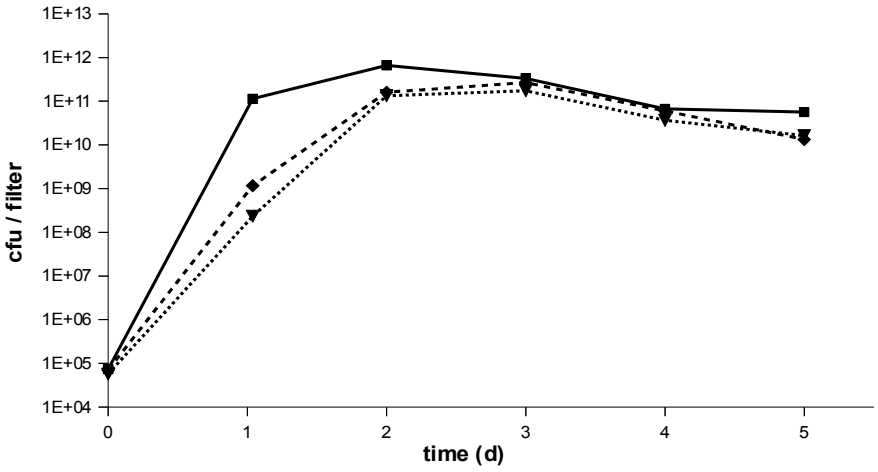

Fig. 29: Anaerobic colony biofilm growth of *Pseudomonas aeruginosa* PAO1, *pfpI*::Is*lacZ*/hah and *pfpI*::Is*phoA*/hah transposon insertion mutants. Colony biofilms of wild type (solid line, squares), *pfpI*::Is*lacZ*/hah transposon insertion mutant #10045 (dashed line, diamonds) and *pfpI*::Is*phoA*/hah transposon insertion mutant #39274 (dotted line, triangles) were grown on Durapore membrane filters resting on LB agar plates (2.5.2) supplemented with 50 mM KNO_3. Colony forming units (cfu/filter) were determined every 24 hours by plating (2.5.4), values represent the average of two independent experiments.

As seen in Fig. 29, anaerobic colony biofilm growth of PAO1 *pfpI*::Is*lacZ*/hah and *pfpI*::Is*phoA*/hah transposon insertion mutant strains was delayed in comparison to wild type. Whereas PAO1 wild type reaches approximately 1 x 10^{11} cfu/filter after one day, both transposon insertion mutant strains show to approximately 1 x 10^{9} cfu/filter. However, after three days of incubation *pfpI*::Is*lacZ*/hah and *pfpI*::Is*phoA*/hah transposon insertion mutant strains have reached colony forming units similar to wild type. Since this phenotype does not resemble that observed for Δ*relA*Δ*spoT,* Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutants strains (3.1.2), it is unlikely that a deregulation of *pfpI* expression is responsible for impaired colony biofilm growth of ppGpp- or DksA-deficient strains.

In conclusion, although expression of several genes previously reported to contribute to *P. aeruginosa* biofilm growth was controlled by the stringent response, the absence of most of these factors did not negatively affect anaerobic colony biofilm growth. An exception was a the deletion of σ^S-encoding *rpoS* which resulted in a reduced number of colony forming units during anaerobic colony biofilm growth, although the effect was not nearly as pronounced as observed for Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutants strains (3.1.2). However, as deletion of *nirS* partly restored anaerobic colony biofilm growth of mutant strains unable to synthesize ppGpp or DksA (3.1.3), it is possible that an interplay of nitric oxide mediated damage and a deregulation of *rpoS* is responsible for the growth defects of Δ*relA*Δ*spoT*, Δ*dksA* and Δ*relA*Δ*spoT*Δ*dksA* mutants strains during anaerobic colony biofilm growth.

3.2 Adaptation of *Pseudomonas putida* to oxygen limitation

In contrast to *Pseudomonas aeruginosa* or *Pseudomonas stutzeri, Pseudomonas putida* KT2440 is an obligate aerobic bacterium and thus unable to grow anaerobically. However, both *P. putida* and facultative anaerobic bacteria such as *P. aeruginosa*, are able to respond oxygen limitation via the global oxygen-sensing regulator Anr. So far the Anr regulons of closely related obligate aerobic and facultative anaerobic bacteria have not been compared. In this study Anr-dependent gene expression in *P. putida* KT2440 upon oxygen depletion was investigated and compared to the published Anr regulon of *P. aeruginosa* (Trunk *et al.*, 2010). Since a shift to anaerobic conditions is also accompanied by an energy starvation, the role of the stringent response in *P. putida* during adaptation to oxygen-limiting conditions was also investigated. As seen in the first part of this study, the stringent response plays an important role in *P. aeruginosa* anaerobic survival (3.1.1), so ppGpp might contribute to *P. putida* survival in the absence of oxygen as well.

P. aeruginosa grows anaerobically by respiration of nitrate or nitrite. It was investigated whether the respective *P. aeruginosa* PAO1 gene clusters encoding enzymes required for denitrification promote anaerobic growth of *P. putida* KT2440. Effects of the transferred *P. aeruginosa* operons on *P. putida* gene expression profiles during anaerobiosis were monitored by transcriptome analysis.

3.2.1 Annotation of previously uncharacterized *Pseudomonas putida* KT2440 open reading frames

Various *P. putida* KT2440 expression profiles should be investigated via microarray analysis with custom-designed Agilent gene expression oligo microarrays in this work. Initially, it was observed that several genes highly conserved among Pseudomonads are not annotated in KT2440. For instance, the ribosome modulation factor encoding *rmf* gene, which is present in all sequenced Pseudomonads including *P. putida* strains F1, GB-1 and W619, is not annotated in *P. putida* KT2440. Fig. 30 depicts a schematic representation with *P. putida* F1 gene Pput_3644, encoding the ribosome modulation factor, id its genomic context compared to the corresponding region in KT2440 genome according to "*Pseudomonas* Genome Database" (Winsor *et al*., 2009).

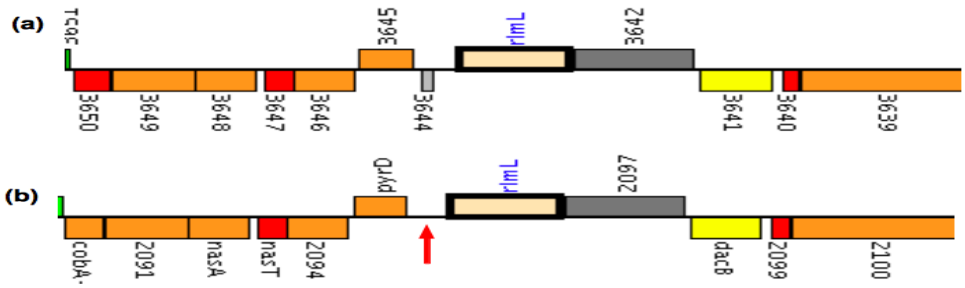

Fig. 30: Differences in genome annotations of *Pseudomonas putida* F1 and *Pseudomonas putida* KT2440, the latter lacking an ortholog encoding a ribosome modulation factor. (a) *Pseudomonas putida* F1 genome from position 4081300 to 4065300 (orientation flipped) including gene Pput_3644, encoding the ribosome modulation factor, and (b) KT2440 genome from position 2382100 to 2397500, without an annotated gene encoding the ribosome modulation factor (red arrow). Genomic organization according to "*Pseudomonas* Genome Database".

BLASTN analysis with the sequence of *P. putida* F1 Pput_3644 revealed a sequence of 100 % nucleotide identity in the KT2440 genome, located in the same genomic context in which the *rmf* gene is located in other Pseudomonads (data not shown). To verify these findings northern blot analysis (Benkert *et al*., 2008) with a probe designed against *P. aeruginosa* PAO1 *rmf* available in the laboratory was carried out. As nucleotide sequences of PAO1 *rmf* and putative KT2440 *rmf* encoding sequence share 77 % percent identity, a hybridization of a PAO1 *rmf* probe to potential KT2440 *rmf* transcripts is likely. It is known from other bacteria that *rmf* gene expression is induced by the stringent response upon stationary phase entry (Izutsu *et al*., 2001). Consequently, total RNA was extracted from a KT2440 overnight culture was used to detect potential *rmf* transcripts. Indeed, a transcript of approximately 350 bp was detected via northern blot analysis (data not shown). The genome sequence encoding the putative KT2440 *rmf* gene consists of 213 bp, which in combination with the 5' untranslated region (UTR) results in a transcript of approximately 350 bp. Combined with results from BLASTN analysis these findings strongly suggest KT2440 possesses a functional *rmf* gene not annotated in the current version of "*Pseudomonas* Genome Database".

In a next step it was investigated if other ORF's are missing in the KT2440 genome annotation provided by "*Pseudomonas* Genome Database". For that purpose the KT2440 genome sequence was analyzed with "GLIMMER3" (2.10.2), a software designed to specifically identify microbial ORF's.

Besides 5420 currently annotated ORF's, 408 additional putative coding regions were identified by "GLIMMER3". These putative ORF's were further investigated by analysis of their genomic context, such as their overlap with other coding regions, terminator sequences or inverted repeats. Additionally, a BLASTP search of the translated protein

sequences was carried out in order to identify homologous proteins in other bacteria and an assigned protein function.

Based on this analysis 129 additional putative ORF's were identified for *P. putida* KT2440. If all of these are indeed coding regions KT2440 actually possesses 5549 ORF's, implicating that 2.3 % of the genome is currently not annotated. Newly identified ORF's were assigned in the “Wikiputida” database, named after their preceding gene with appendix “.1”. If more than one newly annotated gene followed, appendices “.2” or “.3” were used.

BLASTP analysis revealed that 105 of 129 (81 %) newly identified gene products share highest homologies to hypothetical or conserved hypothetical proteins of other bacteria, often Pseudomonads. However, 24 newly identified ORF's share highest homologies to proteins with assigned functions, such as *P. putida* F1 diguanylate phosphodiesterase (PP2207.1), *Pseudomonas entomophila* L48 LuxR family DNA binding protein (PP1546.1) or *Marinomonas sp*. MWYL1 transcriptional regulator (PP2238.1). Appendix E enlist newly annotated KT2440 genes, including their best matches in BLASTP alignments.

The KT2440 genome deposited in the “Wikiputida” database including 129 additional ORF's identified *in silico* was used to design Agilent gene expression oligo microarrays for transcriptome analysis. KT2440 genes encoding rRNA's and tRNA's were also included on the microarray. For transcriptome analysis carried out in 3.2.3, the microarray also contains probes against *P. aeruginosa* PAO1 gene clusters PA3871 – PA3880, PA0509 – PA0527.1 and PA3390 – PA3396 encoding the *P. aeruginosa* PAO1 gene clusters involved in denitrification via nitrate, nitrite, nitric oxide and nitrous oxide reduction, respectively.

3.2.2 Adaptation of *Pseudomonas putida* to oxygen depletion via Anr and ppGpp

3.2.2.1 *In silico* analysis of the *Pseudomonas putida* Anr regulon

In a first step to identify KT2440 promoters regulated by Anr *in silico* analysis using the “Virtual Footprint – Regulon Analysis” software was carried out (2.10.1). This software identifies sequence homologies of known regulator binding sites in bacterial genomes, in case of Anr based on experimentally determined *P. aeruginosa* PAO1 Anr binding sites (Trunk *et al.*, 2010; Münch *et al.*, 2005). As *P. putida* and *P. aeruginosa* Anr share 88 % amino acid identity, they likely recognize similar promoter binding sites. To investigate the differences and similarities of KT2440 and *P. aeruginosa* PAO1 Anr regulons, the latter was also determined by *in silico* analysis. 239 promoters with a putative Anr binding domain were identified in *P. putida* KT2440, 169 promoters in the *P. aeruginosa* PAO1

genome. Fig. 31 depicts a functional classification of KT2440 and PAO1 genes whose promoters were identified as Anr-dependent by “Virtual Footprint – Regulon Analysis”.

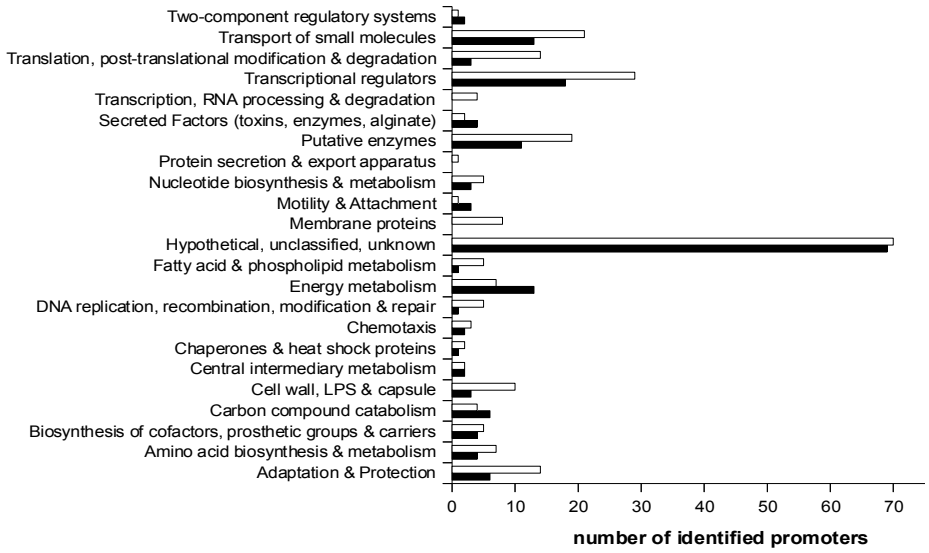

Fig. 31: Functional classification of *Pseudomonas putida* KT2440 (white bars) and *Pseudomonas aeruginosa* PAO1 (black bars) genes under control of promoters carrying a putative Anr binding domain. Promoters were identified with “Virtual Footprint – Regulon Analysis” software with a single pattern search for position weight matrix “Anr_Dnr” of *P. aeruginosa* PAO1 (2.10.1). Classification categories are chosen according to “*Pseudomonas* Genome Database”.

As seen in Fig. 31, for both *P. putida* and *P. aeruginosa* a large number of genes encoding transcriptional regulators were predicted as Anr-dependent by *in silico* analysis. In KT2440 the majority of these encode regulators with no assigned function, except for PP1773 which encodes a putative beta subunit of DNA-binding protein IHF and *phhR*, which encodes a transcriptional activator essential for phenylalanine degradation (Herrera *et al*., 2010). Transcriptional regulator encoding gene PP4470, which is an ortholog of *P. aeruginosa amrZ,* was also predicted to be regulated via Anr. In *P. aeruginosa* PAO1 AmrZ was shown to activate the expression of *algD* involved in alginate biosynthesis as well as to be required for transcriptional control of genes involved in type IV pilus localization and twitching motility (Baynham *et al.*, 2006). Although *P. putida* KT2440 possesses homologs of nearly all genes which were shown to be required for alginate biosynthesis in *P. aeruginosa* (Nelson *et al.*, 2002; Ramsey & Wozniak, 2005), its ability to produce alginate has not been demonstrated *in vivo*. However, it was shown that the parent strain of KT2440, *P. putida* mt-2, produces alginate and other exopolysaccharides in response to water-limiting conditions (Chang *et al.*, 2007). Therefore, a potential Anr-dependent regulation of gene PP4470 might affect alginate biosynthesis as well as type IV pili biosynthesis, the latter possibly contributing to *P. putida* twitching motility in order to escape from anaerobic milieus.

Among the genes of *P. aeruginosa* PAO1 promoters predicted as Anr-dependent are several regulatory factors involved in the onset of anaerobic energy metabolism (*dnr*, *nosR*, *narX*). *P. aeruginosa* Dnr recognizes promoter binding sites indistinguishable from Anr and the *nosR* promoter was shown to be specifically recognized by Dnr and not Anr (Arai *et al.*, 2003). A Dnr- rather than an Anr-dependency is also true for other *P. aeruginosa* promoters identified as regulated by Anr via *in silico* analysis, which was previously verified by experimental data (Trunk *et al.*, 2010).

In both *P. putida* and *P. aeruginosa* various genes encoding for transporters of small molecules and factors involved in energy metabolism were identified as Anr-dependent via regulon analysis. As expected factors participating in denitrification, arginine and pyruvate fermentation are proposed to be regulated by Anr in *P. aeruginosa*. The KT2440 *arcD* promoter, encoding a putative arginine/ornithine antiporter, was also identified as Anr-dependent, providing a first hint *P. putida* KT2440 is able to ferment arginine in the absence of oxygen.

In contrast to *P. aeruginosa* a larger number of promoters of genes involved in adaption and protection were predicted to carry a putative Anr box in KT2440. The majority of these genes belong to universal stress protein (*usp*) family and are orthologs of *P. aeruginosa uspL*, *uspM* and *uspN*, which exist in several copies in the KT2440 genome. An Anr-dependent expression of PAO1 *uspL*, *uspM* and *uspN* during anaerobic conditions was previously determined (Boes *et al.*, 2006; Boes *et al.*, 2008).

In order to identify other genes which were predicted as Anr-dependent by “Virtual Footprint – Regulon Analysis” in *P. putida* as well as *P. aeruginosa,* determined regulons were compared in detail. All ortholog genes whose promoters were predicted to carry an Anr binding site in both KT2440 and PAO1, were identified in order to establish a core Anr regulon for obligate aerobic and facultative anaerobic Pseudomonads (Table 12).

Table 12: Ortholog genes whose promoters were *in silico* predicted as regulated by oxygen-sensing transcriptional regulator Anr in both *Pseudomonas putida* KT2440 and *Pseudomonas aeruginosa* PAO1. Promoters were identified with “Virtual Footprint – Regulon Analysis” based on sequence homologies to the *P. aeruginosa* PAO1 Anr sequence motif. Details on search criteria and information on obtained PWM scores, ATG distances and positions of identified promoter regions are described elsewhere (2.10.1 and Appendix F). Results a sorted by PP numbers of identified KT2440 genes, function of gene products according to “*Pseudomonas* Genome Database”.

***P. putida* KT2440**		***P. aeruginosa* PAO1**	
PP0072 (*qor-1*)	quinone oxidoreductase	PA0023 (*qor*)	quinone oxidoreductase
PP0073 (*hemF*)	coproporphyrinogen-III oxidase, aerobic	PA0024 (*hemF*)	coproporphyrinogen III oxidase, aerobic
PP0202	CBS domain protein	PA0250	conserved hypothetical protein

PP0273	putative uncharacterized protein	PA0200	hypothetical protein
PP0504 (*oprG*)	outer membrane protein OprG	PA4067 (*oprG*)	outer membrane protein OprG
PP0910	putative uncharacterized protein	PA4371	hypothetical protein
PP1002 (*arcD*)	arginine/ornithine antiporter	PA5170 (*arcD*)	arginine/ornithine antiporter
PP1288 (*algD*)	GDP-mannose 6-dehydrogenase	PA3540 (*algD*)	GDP-mannose 6-dehydrogenase
PP2187, PP2745	universal stress protein family	PA1789 (*uspL*)	universal stress protein
PP2188	tRNA-(Ms(2)io(6)a)-hydroxylase	PA1790	hypothetical protein
PP2310	methyl-accepting chemotaxis transducer	PA2867	probable chemotaxis transducer
PP2322 (*oprI*)	outer membrane lipoprotein OprI	PA2853 (*oprI*)	outer membrane lipoprotein OprI precursor
PP2648	universal stress protein family	PA4352 (*uspN*)	universal stress protein
PP3232	acetyltransferase, GNAT family	PA5475	hypothetical protein
PP3237, PP3288 PP3290, PP3294	universal stress protein family	PA4328 (uspM)	universal stress protein
PP4009 (*clpS*)	ATP-dependent Clp protease	PA2621	conserved hypothetical protein
PP4010 (*cspD*)	cold-shock protein CspD	PA2622 (*cspD*)	cold-shock protein CspD
PP4264 (*hemN*)	oxygen-independent coproporphyrinogen III oxidase	PA1546 (*hemN*)	oxygen-independent coproporphyrinogen III oxidase
PP4434 (*dadA1*)	D-amino acid dehydrogenase 1 small subunit	PA5304 (*dadA*)	D-amino acid dehydrogenase, small subunit
PP4470 (*algZ*)	alginate biosynthesis transcriptional activator	PA3385 (*amrZ*)	alginate and motility regulator Z
PP4651 (*cioA*)	ubiquinol oxidase subunit I, cyanide insensitive	PA3930 (*cioA*)	cyanide insensitive terminal oxidase
PP4870	azurin	PA4922 (*azu*)	azurin precursor
PP4871	putative uncharacterized protein	PA4923	conserved hypothetical protein
PP5206	membrane fusion protein	PA5232	conserved hypothetical protein

As seen in Table 12, 24 promoters of ortholog genes were predicted to be Anr-dependent in both *P. putida* KT2440 and *P. aeruginosa* PAO1. Noticeably, an overlap in regulation of key factors involved in energy metabolism was observed, for instance both oxygen-dependent and oxygen-independent coproporphyrinogen III oxidases (*hemN*, *hemF*) participating in heme biosynthesis, quinone oxidoreductase (*qor1*) or cyanide insensitive terminal oxidase (*cioA*). The *arcD* gene encoding an arginine/ornithine antiporter required for arginine fermentation, was also identified as Anr-dependent in both KT2440 and PAO1. Additionally, various promoters of Usp encoding genes were predicted to be regulated via Anr in both *P. putida* and *P. aeruginosa*, as well as cold shock protein encoding gene *cspD*, also contributing to the cellular stress response. The *in silico* determined overlap in Anr-dependent gene regulation in KT2440 and PAO1 also features two genes encoding outer membrane proteins (*oprG*, *oprI*). *P. aeruginosa* OprG was previously shown to be dependent on Anr (McPhee *et al*., 2009). Lastly, two promoters of genes contributing to algniate biosynthesis (*algD*, *algZ*) were predicted to contain an Anr

binding site in KT2440 as well as PAO1. Although KT2440 has not been characterized regarding its ability to produce alginate, an increased production of alginate by *P. aeruginosa* during anaerobic conditions has been previously reported (Hassett, 1996).
Although determination of a core Anr regulon for *P. putida* and *P. aeruginosa* revealed a common set of genes only 10 % of KT2440 genes putatively regulated via Anr were also predicted as Anr-dependent in PAO1. These findings suggest two highly specific Anr regulons of obligate aerobic strain *P. putida* and facultative anaerobic strain *P. aeruginosa,* adjusted to their respective metabolic abilities in the absence of oxygen. Interestingly, although Anr is predicted to regulate a large number of genes encoding transcriptional regulators in both bacteria, with the exception of alginate biosynthesis transcriptional activator (KT2440 *algZ* and PAO1 *amrZ*, respectively), none of these genes are orthologous. These findings further demonstrate the differences of the respective Anr regulons of *P. aeruginosa* and *P. putida*.
P. aeruginosa PAO1 genes of promoters predicted to be regulated in an Anr-dependent manner were described previously (Trunk *et al*., 2010), Appendix F lists all *P. putida* KT2440 genes with putative Anr binding sites.

3.2.2.2 Contribution of Anr and the stringent response to *Pseudomonas putida* survival in the absence of oxygen

In contrast to related bacteria like *P. aeruginosa* or *P. stutzeri*, *P. putida* does not carry genes encoding for anaerobic growth with nitrate as an alternative electron acceptor. Therefore, determining survival of *P. putida* during oxygen limitation requires the application of anaerobic shift experiments (2.5.2). To investigate the role of global oxygen-sensing regulator Anr (1.2.2.1) in adaptation of KT2440 to oxygen depletion, a KT2440 Δ*anr* mutant strain was constructed. A KT2440 Δ*relA*Δ*spoT* mutant strain was generated to determine the involvement of the stringent response (1.3) in adaptation of *P. putida* to the absence of oxygen.
In order to investigate whether the absence of Anr or ppGpp affects *P. putida* survival during oxygen depletion, anaerobic shift experiments were carried out. Fig. 32 depicts survival rates of Δ*anr*, Δ*relA*Δ*spoT* and wild type after transition from aerobic growth to oxygen-depleted conditions.

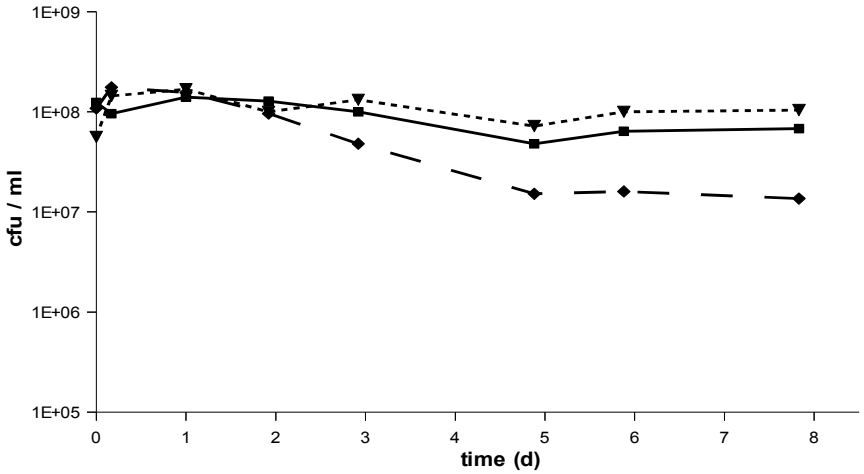

Fig. 32: Survival of *Pseudomonas putida* KT2440 and mutant strains Δ*anr* and Δ*relA*Δ*spoT* after transition from mid-exponential aerobic growth to oxygen-depleted conditions. Planktonic cultures of wild type (solid line, squares), Δ*anr* (dashed line, diamonds) and Δ*relA*Δ*spoT* (dotted line, triangles) were grown to an OD_{578} of 0.5 in LB and shifted to oxygen-depleted conditions by transfer of the culture in sealed serum flasks. Survival rates (cfu/ml) were determined by plating (2.5.4) at time points indicated.

As seen in Fig. 32, KT2440 wild type survives up to one week after the anaerobic shift with no significant decrease in the number of colony forming units. Whereas the Δ*relA*Δ*spoT* mutant strain shows survival rates similar to the wild type, colony forming units observed for Δ*anr* mutant strain decrease more rapidly after three days of incubation and onward. After eight days of incubation approximately 1 x 10^7 cfu/ml were detectable for Δ*anr* mutant strain, compared to approximately 1 x 10^8 cfu/ml detected for wild type. These results clearly demonstrate oxygen-sensing regulator Anr is essential for KT2440 long-term survival during oxygen-depleted conditions. A role of the stringent response in adaptation to oxygen limitation was not observed in this experimental setup as the Δ*relA*Δ*spoT* mutant strain behaved wild type-like.

In a next step changes in gene expression of *P. putida* KT2440 Δ*anr*, Δ*relA*Δ*spoT* and wild type in response to oxygen depletion were investigated by transcriptome analysis with custom-designed *P. putida* Agilent gene expression oligo microarrays. Details on experimental procedures of transcriptome analysis with Agilent microarrays, in particular the differences to *P. aeruginosa* Affymetrix GeneChips (2.9.1) applied in the first part of this study (3.1.4), are described elsewhere (2.9.2).

In order to investigate the transcriptional response of KT2440 Δ*anr*, Δ*relA*Δ*spoT* and wild type to oxygen depletion, anaerobic shift experiments were carried out. It was shown previously that residual oxygen after the anaerobic shift is consumed within five minutes via aerobic respiration (Eschbach *et al.*, 2004). To monitor the response to oxygen depletion cells were grown aerobically to an OD_{578} of 0.5 and transferred to oxygen-

impermeable sealed serum flasks. Cells for microarray analysis were harvested prior to the anaerobic shift and after 30 minutes of oxygen depletion. Total RNA was prepared (2.6.11), analyzed with Agilent 2100 Bioanalyzer and transcriptome analysis was carried out with Agilent gene expression oligo microarrays (2.9.2). Raw microarray data was preprocessed, fold change expression values calculated (2.9.2) and transcriptome profiles were analyzed according to the diagram depicted in Fig. 33.

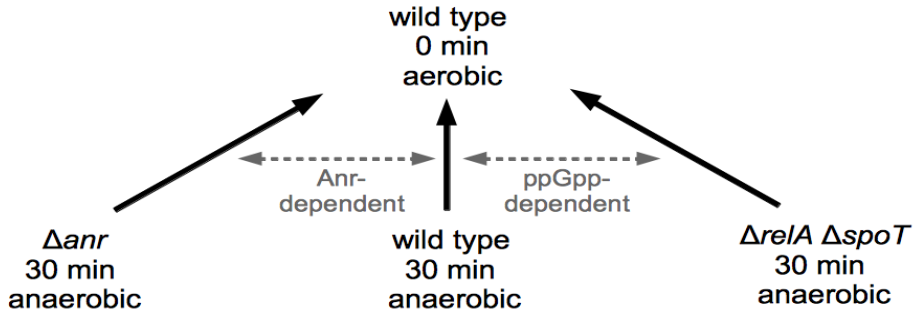

Fig. 33: Schematic overview of transcriptome data analysis for investigation of *Pseudomonas putida* KT2440 Anr- and ppGpp-dependent gene regulation in response to oxygen depletion. Experimental procedures are described in text. Solid arrows indicate two growth conditions compared by the respective two-dye Agilent gene expression oligo microarrays carried out in triplicate. Dotted arrows indicate *in silico* comparisons in order to identify Anr- and ppGpp-dependent genes. Transcriptome profiles of KT2440 wild type cells prior to anaerobic shift served as reference sample. Expression profiles obtained for KT2440 wild type after 30 minutes of oxygen depletion were compared to those of Δ*anr* and Δ*relA*Δ*spoT* mutant strains after 30 minutes of oxygen depletion.

Transcriptome analysis revealed 454 differentially expressed genes in KT2440 wild type after 30 minutes of oxygen depletion compared to wild type prior to the anaerobic shift. Of these genes, 186 were induced and 268 were repressed more than 2-fold in the absence of molecular oxygen. In order to identify which of the differentially expressed genes are Anr- or ppGpp-dependent, expression profiles of Δ*anr* and Δ*relA*Δ*spoT* mutant strains after 30 minutes of oxygen limitation were compared to those of KT2440 wild type.

3.2.2.3 Anr-dependent gene expression in *Pseudomonas putida* in response to oxygen depletion

Microarray analysis revealed 197 genes differentially regulated in wild type, but not Δ*anr* mutant strain, after 30 minutes of oxygen depletion, representing genes directly or indirectly regulated by Anr. Of these genes, 104 were induced and 93 repressed by Anr. Appendix G lists all *P. putida* KT2440 genes differentially regulated by Anr after 30 minutes of oxygen-depleted incubation compared to wild type prior to the anaerobic shift and their respective fold changes. Fig. 34 depicts a functional classification of

identified genes.

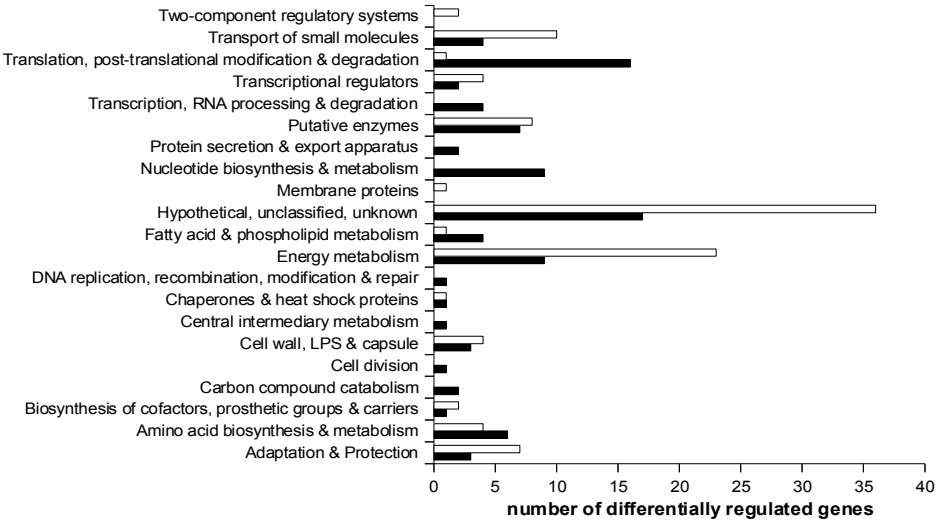

Fig. 34: Functional classification of *Pseudomonas putida* KT2440 genes differentially regulated in wild type, but not Δ*anr* mutant strain, after 30 minutes of oxygen depletion. Experimental procedures and data analysis are described elsewhere (3.2.2.2). Classification categories are chosen according to "*Pseudomonas* Genome Database". Depicted are genes (white bars) induced or (black bars) repressed in wild type, but not Δ*anr* mutant strain, after 30 minutes of oxygen depletion compared to wild type prior to the anaerobic shift.

As seen in Fig. 34, Anr induces a number of genes participating in energy metabolism in response to oxygen depletion, for instance genes encoding cytochrome bc_1 complex (*petAB*), cytochrome *c* (e. g. PP0125, PP5267, *ccmCEF*), azurin (PP4870) or cbb3-1 type cytochrome *c* oxidase ($ccoN_1O_1P_1Q_1$). An Anr-dependent upregulation of genes $ccoN_1O_1P_1Q_1$ during low oxygen conditions was previously reported (Ugidos *et al.*, 2008). Expression of the *arcCIAD* operon, involved in a putative arginine fermentation, is also induced in an Anr-dependent manner upon oxygen depletion, suggesting *P. putida* KT2440 might indeed be able to gain energy by this fermentation in the absence of molecular oxygen.

P. putida Anr also induces several genes involved in adaptation and protection in response to oxygen limitation. Prominently featured in this category are genes encoding universal stress proteins, i. e. PP2187, PP2648, PP3288, PP3290 and PP3237. *P. aeruginosa* orthologs of these genes (*uspL*, *uspM*, *uspN*) were previously shown to be regulated in an Anr-dependent manner in response to anaerobic conditions (Boes *et al.*, 2008).

As seen in Fig. 34, Anr is also required for repression of a number of genes as initial response to oxygen depletion. These findings differ from those obtained for *P. aeruginosa* in a similar anaerobic shift experiments (Trunk *et al.*, 2010), in which no genes were repressed in an Anr-dependent manner. However, as *P. aeruginosa* was incubated for two

hours after the anaerobic shift and offered nitrate for anaerobic respiration, the slight experimental variances might explain the different results obtained for *P. putida*. In order to gain hints if the identified *P. putida* genes are directly downregulated via Anr or indirect effects might be responsible for a repression, their promoter regions were analyzed *in silico* by “Virtual Footprint – Promoter Analysis” with a pattern search for PWM “Anr_Dnr” of *P. aeruginosa* (2.10.1). Table 13 lists all genes for which a putative Anr binding site was identified in the respective promoter region. Note that these genes were not identified as Anr dependent by “Virtual Foorprint – Regulon Analysis” (3.2.2.1), as the identified binding sites are located in coding regions, which were excluded during regulon analysis.

Table 13: *In silico* predicted Anr binding sites of genes, which were experimentally identified as repressed by Anr in *Pseudomonas putida* KT2440 after 30 minutes of oxygen depletion. Experimental procedures and data analysis are described elsewhere (3.2.2.2). Respective promoter regions of genes identified as negatively regulated by Anr in anaerobic shift experiments (Appendix G) were analyzed with “Virtual Footprint – Promoter Analysis” (2.10.1). Genes are sorted by PP number, start/end describe the position of predicted Anr box relative to ATG and PMW score the degree of conservation of predicted Anr box.

PP number	gene name	start	end	PMW score	sequence of predicted Anr box
PP0442	*nusG*	205	218	12.39	TTGTGGGTAATCAA
PP0480	*rplQ*	351	364	12.78	TTGATTTCAGTCAG
		351	364	12.65	CTGACTGAAATCAA
PP0811	*cyoups2*	269	282	13.99	TTGATCTGGATCAA
		269	282	13.97	TTGATCCAGATCAA
		308	321	13.46	TTGAGGCCTATCAA
		308	321	13.39	TTGATAGGCCTCAA
PP0812	*cyoA*	7	20	13.79	TTGATCTGGATCAA
		7	20	13.75	TTGATCCAGATCAA
		46	59	13.26	TTGATAGGCCTCAA
		46	59	13.23	TTGAGGCCTATCAA
PP0933	*mreB*	65	78	12.36	CTGCCATGCATCAA
PP0964	*murA*	355	368	12.50	CTGAGTCCGGTCAA
		325	338	12.50	CTGAGTCCGGTCAA
		325	338	12.38	TTGAACGTGATCAG
		355	368	12.28	TTGACCGGACTCAG
		226	239	11.64	ATGCTCAACCTCAA
PP1594	*frr*	412	425	12.10	TTGCGGGTATTCAA
		253	266	11.49	TTGGTCGCCTTCAG
PP1605	*rnhB*	93	106	12.68	TTGGCGCCCTTCAA
		476	489	11.61	CTGGCCCTGCTCAA
PP1790		73	86	12.75	CTGACATCAATCAA
PP1790		73	86	12.59	TTGATTGATGTCAG

PP number	gene name	start	end	PMW score	sequence of predicted Anr box
PP4715	*tpiA*	136	149	12.87	CTGATGGCGCTCAA
		136	149	12.72	TTGAGCGCCATCAG
		343	356	11.83	TTGGTGCGGGTCAT

As seen in Table 13, for ten genes an Anr binding site at a position indicating a repression by Anr was predicted. Among the experimentally determined Anr-dependent genes with a putative Anr box in their upstream sequence, are *cyoA* and *cyoups2*. Other genes of the *cyoABCDE* operon, encoding a *bo*3-type cytochrome oxidase, were also repressed in an Anr-dependent manner. A negative regulation of genes *cyoABCDE* by Anr was previously described (Ugidos *et al.*, 2008).

Interestingly, two genes involved in translation, encoding a ribosome recycling factor (*frr*) and ribosomal protein L17 (*rplQ*), were experimentally identified as Anr-dependent in *P. putida* and carry a putative Anr binding site in their promoter region. In *P. aeruginosa*, no influence of Anr on genes whose products participate in translation is known.

Although 80 additional genes were downregulated in an apparently Anr-dependent manner, only a few of them contain an Anr binding site in the respective promoter region at a position indicating a repression by Anr. A negative regulation of genes with no putative Anr box in their promoter region in response to oxygen depletion, might be indirectly affected by Anr.

Comparison of experimentally determined *P. putida* KT2440 Anr regulon with *in silico* prediction (3.2.2.1) revealed that 28 of 239 predicted Anr-dependent KT2440 promoters could be experimentally verified as regulated via Anr in response to oxygen depletion. Transcriptome analysis was carried out after 30 minutes of oxygen-limiting conditions, so it is possible that expression of the majority of *in silico* predicted genes is not affected by Anr until a later time point or requires additional signals and regulators as presumed for *P. aeruginosa* (Trunk *et al.*, 2010). Further analysis of this set of genes revealed that the respective predicted Anr boxes obtained high PMW scores during *in silico* analysis, suggesting a high conservation of the identified binding site.

In order to determine similarities of *P. aeruginosa* and *P. putida* Anr-mediated responses to oxygen depletion, the experimentally determined Anr regulon of PAO1 (Trunk *et al.*, 2010) was compared to that determined for KT2440. This comparison revealed that Anr-dependent 18 *P. putida* genes possess *P. aeruginosa* orthologs, which were also identified as regulated by Anr (Table 14).

Table 14: Ortholog genes whose expression was induced by oxygen-sensing transcriptional regulator Anr in both *Pseudomonas putida* KT2440 and *Pseudomonas aeruginosa* PAO1 in response to oxygen limitation. Anr-dependent genes were identified in anaerobic shift experiments during mid-exponential growth phase by comparison of expression profiles obtained for wild types and Δ*anr* mutant strains. Details on experimental procedures and data analysis are described elsewhere (3.2.2.2.), fold change expression values of the respective *P. putida* KT2440 genes are listed in Appendix G. Transcriptome profiles for *P. aeruginosa* were employed from previous studies (Trunk *et al.*, 2010). Gene are sorted by PP numbers of *P. putida* genes, function of gene products according to "*Pseudomonas* Genome Database".

***P. putida* KT2440**		***P. aeruginosa* PAO1**	
PP0273	conserved hypothetical protein	PA0200	hypothetical protein
PP0504 (*oprG*)	outer membrane protein OprG	PA4067 (*oprG*)	outer membrane protein OprG
PP0998	conserved hypothetical protein	PA1076	hypothetical protein
PP0999 (*arcC*)	carbamate kinase	PA5173 (*arcC*)	carbamate kinase
PP1000 (*argI*)	ornithine carbamoyltransferase, catabolic	PA5172 (*arcB*)	ornithine carbamoyltransferase, catabolic
PP1001 (*arcA*)	arginine deiminase	PA5171 (*arcA*)	arginine deiminase
PP1002 (*arcD*), PP1003	arginine/ornithine antiporter	PA5170 (*arcD*)	arginine/ornithine antiporter
PP2187	universal stress protein family	PA1789 (*uspL*)	universal stress protein
PP2648	universal stress protein family	PA4352 (*uspN*)	universal stress protein
PP3232	acetyltransferase, GNAT family	PA5475	hypothetical protein
PP3237, PP3288 PP3290	universal stress protein family	PA4328 (*uspM*)	universal stress protein
PP3839 (*adhA*)	alcohol dehydrogenase, zinc-containing	PA5427 (*adhA*)	alcohol dehydrogenase
PP4251 (*ccoO1*)	cytochrome c oxidase, cbb3-type, subunit II	PA1556 (*ccoO2*)	Cytochrome c oxidase, cbb3-type, CcoO subunit
PP4264 (*hemN*)	oxygen-independent coproporphyrinogen III oxidase	PA1546 (*hemN*)	oxygen-independent coproporphyrinogen III oxidase
PP5207	ABC transporter, ATP-binding protein/permease protein, putative	PA5231	probable ATP-binding/permease fusion ABC transporter

In agreement with *in silico* predicted as Anr-dependent promoters present in in both *P. putida* and *P. aeruginosa* (3.2.2.1), comparison of experimentally determined Anr regulons reveals several *usp* genes induced in an Anr-dependent manner in response to oxygen limitation. The same is true for genes such as outer membrane protein encoding *orpG* or oxygen-independent coproporphyrinogen III oxidase encoding *hemN*. In contrast to *in silico* analysis, a distinct overlap in Anr-dependent genes in KT2440 with those identified in PAO1 was observed for genes involved in a putative arginine fermentation. Besides *arcD*, no gene whose product participates in arginine fermentation was predicted to be regulated via Anr in either KT2440 or PAO1, whereas all genes of the cluster (PP1003, *arcCIAD*) were experimentally shown to be regulated by Anr in *P. putida* as well

as *P. aeruginosa*.

In conclusion, as observed for *in silico* predicted Anr regulons of *P. aeruginosa* and *P. putida*, only a small set of genes was experimentally identified as Anr-dependent in both bacteria. Adaptation to oxygen-limitation via Anr appears to consist of a specific regulon according to the strains' metabolic abilities during oxygen depletion.

P. putida KT2440 genes experimentally determined as Anr-dependent in response to oxygen depletion are listed in Appendix G. Genes which were additionally identified via *in silico* regulon analysis (3.2.2.1) as well as genes whose orthologs were also regulated by Anr in anaerobic shift experiments with *P. aeruginosa,* are indicated.

3.2.2.4 ppGpp-dependent gene expression in *Pseudomonas putida* in response to oxygen depletion

Microarray analysis revealed 233 genes were differentially regulated in wild type, but not Δ*relA*Δ*spoT* mutant strain after 30 minutes of oxygen depletion, representing genes directly or indirectly regulated by the ppGpp-mediated stringent response. Of these genes 85 were induced and 148 repressed by ppGpp. Appendix H lists all *P. putida* KT2440 genes differentially regulated by ppGpp after 30 minutes of oxygen-depleted incubation compared to wild type prior to the anaerobic shift and their respective fold changes. Fig. 35 depicts a functional classification of the identified genes.

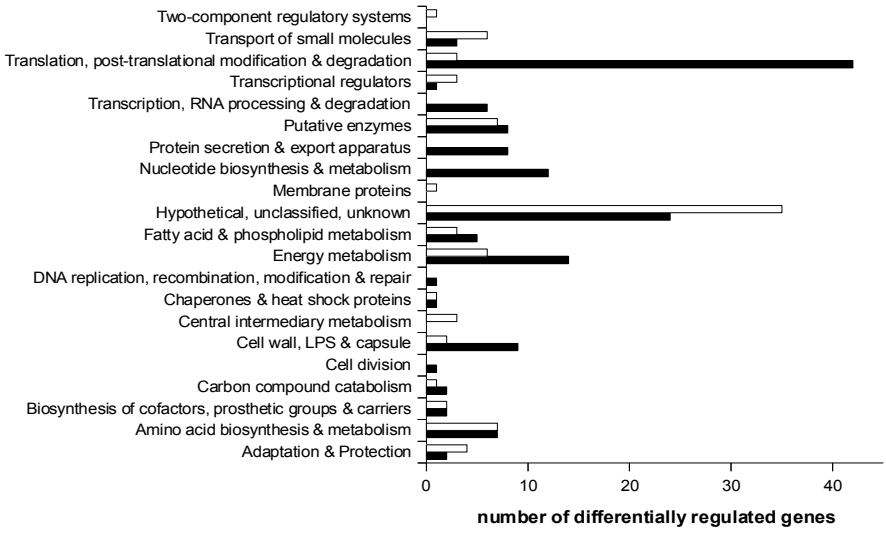

Fig. 35: Functional classification of *Pseudomonas putida* KT2440 genes differentially in wild type, but not Δ*relA*Δ*spoT* mutant strain, after 30 minutes of oxygen depletion. Experimental procedures and data analysis are described elsewhere (3.2.2.2). Classification categories are chosen according to "*Pseudomonas* Genome Database". Depicted are genes (white bars) induced or (black bars) repressed in wild type, but not Δ*relA*Δ*spoT* mutant strain, after 30 minutes of oxygen depletion compared to wild type prior to the anaerobic shift.

As seen in Fig. 35, ppGpp induces several genes involved in energy metabolism in response to oxygen depletion, most noticeably cbb3-1 type cytochrome *c* oxidase encoding *ccoN$_1$O$_1$P$_1$.* Expression of genes *ccoN$_1$O$_1$Q$_1$P$_1$* was shown to be positively affected by Anr under oxygen-limiting conditions in previous reports (Ugidos *et al.*, 2008) as well as in this study (3.2.2.3), but a regulation via the stringent response has not yet been demonstrated. A number of genes contributing to adaptation and protection, such as cold shock protein encoding *cspD,* were upregulated via ppGpp in the anaerobic shift experiment, as well as genes involved in amino acid degradation. Additionally, several genes encoding transporters of small molecules were induced in a ppGpp-dependent manner upon oxygen depletion, including *arcD* and PP1003, both encoding arginine/ ornithine antiporters required for a putative arginine fermentation.

Two genes (*ptsN, ptsP*) encoding compounds of the phosphoenolpyruvate-carbohydrate phosphotransferase transport system (PTS) were induced in a ppGpp-dependent manner in response to oxygen limitation. The PTS mediates phosphorylation and subsequent uptake of a large number of carbohydrates (Postma *et al*., 1993). During carbon overflow conditions many bacteria synthesize polyhydroxyalkanoates (PHA) as carbon and energy reserve materials. PHA's are composed of 3-hydroxyacid monomer units which exist as a small number of cytoplasmic granules per cell (Anderson & Dawes, 1990). It was shown that deletions in genes encoding the *P. putida* PTS affected cellular PHA levels (Velázquez *et al*., 2007). In agreement with a positive control of genes *ptsN* and *ptsP*, ppGpp also induces genes PP5007 and PP5008 encoding PHA granule-associated proteins in response to oxygen-limiting conditions.

In response to oxygen depletion the stringent response is also required for induction of gene *turB,* encoding a MvaT class transcriptional regulator. MvaT, which shares amino acid homologies to H-NS (histone-like nucleoid structuring) proteins, was shown to regulate a number of cellular processes in *P. aeruginosa*. Among these processes are regulation of genes contributing to virulence (Diggle *et al.*, 2002) and synthesis of adhesive structures required for biofilm formation (Vallet *et al.*, 2004) as well as repression of AHL-dependent quorum sensing at low cell densities (Venturi, 2006). MvaT directly interacts with MvaU (Vallet-Gely *et al*., 2005), an interaction which was shown to be required for pyocyanine biosynthesis, repression of the *aotJQMOP-argR* operon mediating arginine uptake and transcriptional silencing of Pf4 prophage genes in *P. aeruginosa* (Li *et al*., 2009). *P. aeruginosa mvaU* is orthologous to *P. putida turB*, which was found upregulated by ppGpp in the anaerobic shift experiment. Transcriptome analysis of *P. putida* KT2440 during both mid-exponential and early stationary phase revealed a TurB-dependent regulation of several genes (i. e. *fadDx*, *atpC*, *secY*, *carB*) (Renzi *et al.*, 2010), which where identified as ppGpp-dependent upon oxygen depletion in

this study. Interestingly, TurB also mediated a 2-fold downregulation of *spoT* expression during early stationary phase, suggesting TurB may induce its own expression by decreasing cellular SpoT levels.

As seen in Fig. 35, ppGpp represses large number of genes involved in translation and nucleotide biosynthesis. Expression of a large number of factors participating in energy metabolism is also downregulated via ppGpp in response to oxygen depletion, including the *bo*3-type cytochrome oxidase encoding operon *cyoABCDE*, whose expression was previously reported to decrease strongly in stationary phase (Morales *et al.*, 2006) during which ppGpp accumulates. Additionally, a large number of genes involved in protein secretion and cell wall biosynthetic processes in a ppGpp-dependent manner in *P. putida*.

3.2.2.5 Concurrence of Anr- and ppGpp-mediated gene expression in *Pseudomonas putida* in response to oxygen depletion

Analysis of transcriptome data revealed a large number of genes whose expression was controlled by both oxygen-sensing regulator Anr and the alarmone ppGpp in anaerobic shift experiments. A co-regulatory system consisting of Anr and the stringent response was shown previously in *P. aeruginosa* for expression of anaerobic *usp* genes contributing to anaerobic survival (Boes *et al.*, 2008). In order to identify genes whose expression was controlled by both Anr and ppGpp in *P. putida* KT2440 in response to oxygen depletion, regulons obtained from transcriptome analysis (3.2.2.2) were cross-examined (Fig. 36).

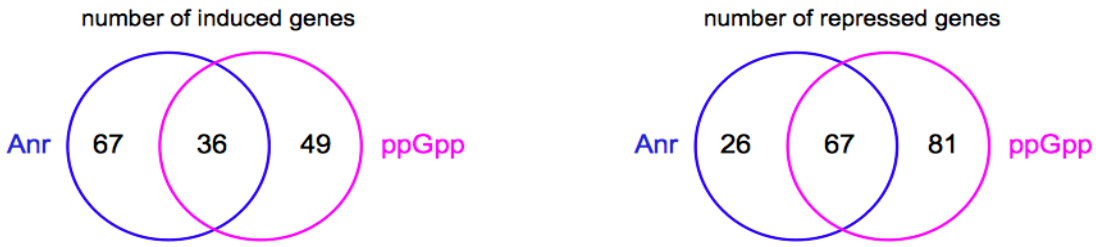

Fig. 36: Venn diagrams of genes differentially regulated by Anr and/or ppGpp in *Pseudomonas putida* KT2440 after 30 minutes of oxygen depletion. Experimental procedures and data analysis are described elsewhere (3.2.2.2), all genes differentially regulated by Anr and ppGpp are listed in Appendix G and H, respectively. Depicted are number of genes (left) induced or (right) repressed by Anr and/or ppGpp.

As seen in Fig. 36, Anr and/or ppGpp differentially regulate a total number of 326 genes in response to oxygen depletion. Of these genes 152 were induced and 174 repressed by Anr and/or ppGpp.

As discussed before both Anr and ppGpp coordinate expression of genes involved in

energy metabolism, such as terminal oxidases (*ccoN$_1$O$_1$P$_1$, cyoABCDE*), or adaptation and protection, such as Usp encoding genes (PP2187, PP3288). Anr and ppGpp both induce several genes encoding transporters of small molecules e. g. *arcD* and PP1003, both mediating arginine/ornithine antiport required during arginine fermentation. Additionally, a coordinated downregulation of several genes involved in amino acid metabolism as well as nucleotide biosynthesis by both Anr and the stringent response was observed.

Four genes encoding transcriptional regulators or two-component regulatory systems were differentially regulated in an Anr- as well as ppGpp-dependent manner. Both Anr and the stringent response upregulate expression of the *ptxS* gene upon oxygen depletion. In *P. aeruginosa* PtsX was shown to mediate activation of the 2-ketogluconate utilization (*kgu*) operon (Swanson *et al.*, 2000), whose products convert 2-ketogluconate to 6-phosphogluconate, which then enters the Entner-Dourdoroff pathway.

Also induced by Anr and ppGpp 30 minutes after the anaerobic shift, are genes *pprA* and *pfrA*, encoding orthologs of *P. aeruginosa algR* and *algQ*, respectively. Transcriptional regulators AlgR and AlgQ were originally identified as positive regulators of the *P. aeruginosa* alginate biosynthetic pathway (Deretic & Konyecsni, 1989; Kato *et al*., 1989) but also control other cellular processes such as exerting a negative regulation on pyoverdine biosynthesis (Lizewski *et al*., 2002; Ambrosi *et al*., 2005). AlgQ was also shown to negatively modulate the expression of quorum sensing regulatory genes *lasR* and *rhlR* (Ledgham *et al.*, 2003). KT2440 *pfrA* encoding an anti-RNAP σ^{70} factor, was 6.5-fold induced upon oxygen depletion in KT2440 wild type but not Δ*anr* or Δ*relA*Δ*spoT* mutant strains. In agreement with these findings, expression of the *pfrA* ortholog *algQ* was also shown to be positively affected via the stringent response in *P. aeruginosa* PAO1 (3.1.4.4). However, no effect of *P. aeruginosa* Anr on *algQ* expression was observed in anaerobic shift experiments (Trunk *et al*., 2010).

Expression of *pprA* was 3.5-fold upregulated in KT2440 wild type but not Δ*anr* or Δ*relA*Δ*spoT* mutant strains after 30 minutes of oxygen depletion. In *P. putida* mt-2 PprA was shown to act as a repressor on the σ^{54}-dependent promoter of TOL plasmid encoded operon *xylUWCMABN* (Vitale *et al*., 2008), whose products mediate degradation of toluene to benzoate (Ramos *et al*., 1997). Oxygen depletion was shown to decrease expression of *xylUWCMABN* as well as *xylXYZLTEGFJQKIH*, the latter encoding factors required for the conversion of benzoate to TCA cycle intermediates (Martínez-Lavanchy *et al*., 2010). These findings suggest that upon oxygen limitation Anr and the stringent response mediate inhibition of the plasmid-encoded *P. putida* toluene degradative pathway by upregulating *pprA* expression, resulting in a subsequent repression of the TOL operons via PprA. Interestingly, ppGpp also induces the *ptsN* gene, encoding part of the

PTS system (3.2.2.4), whose gene product was shown to downregulate *xylUWCMABN* expression in cells growing in a chemostat with succinate (Aranda-Olmedo *et al*. 2006). In agreement with a downregulation of the toluene utilization pathway in response to oxygen depletion, Anr also represses several genes (e. g. *ttg2C*, *ttg2F*) encoding Ttg (toluene tolerance genes) organic solvent efflux pumps which prevent accumulation of aromatic hydrocarbons in cell membranes (Ramos *et al*., 1998; Mosqueda & Ramos, 2000).

Anr and ppGpp both act as repressors of PP0841, encoding a BadM/Rrf2 family transcriptional regulator. Although no information is available on PP0841, it is an ortholog of *P. aeruginosa iscR* and located in a similar genomic context. In *E. coli* IscR was shown to act a repressor of genes *iscSUA-hscBA-fdx* (Schwartz *et al*., 2001) which encode factors of the Isc (iron-sulphur-cluster) system. The Isc system mediates construction of nascent FeS clusters on a scaffold protein and transfers them into recipient proteins. IscR responds to iron availability and oxidative stress conditions in *E. coli* (Giel *et al.*, 2006), consistent with the fact *P. aeruginosa iscR* is important for catalase A activity and peroxide resistance of strain PA14 (Kim *et al*., 2009). It was shown that the *P. aeruginosa* oxidative stress response is induced upon oxygen limitation (Trunk *et al*., 2010), although the reason for this activation remains unknown. An Anr- and ppGpp-dependent downregulation of *iscR* ortholog PP0841 in KT2440 upon anaerobiosis is in agreement with these observations, as IscR was shown to repress genes encoding structural genes of the Isc system (Schwartz *et al*., 2001).

As seen in this chapter several genes differentially regulated in *P. putida* KT2440 in response to oxygen depletion are regulated by oxygen-sensing regulator Anr and the ppGpp-mediated stringent response. Both factors are required for adaptation of the bacterium's metabolism to oxygen-limiting conditions, which are characterized by energy starvation leading to lower growth rates or even resulting in a growth arrested state. Fig. 37 summarizes the proposed Anr- and ppGpp-dependent regulatory network activated in *P. putida* KT2440 in response to oxygen depletion.

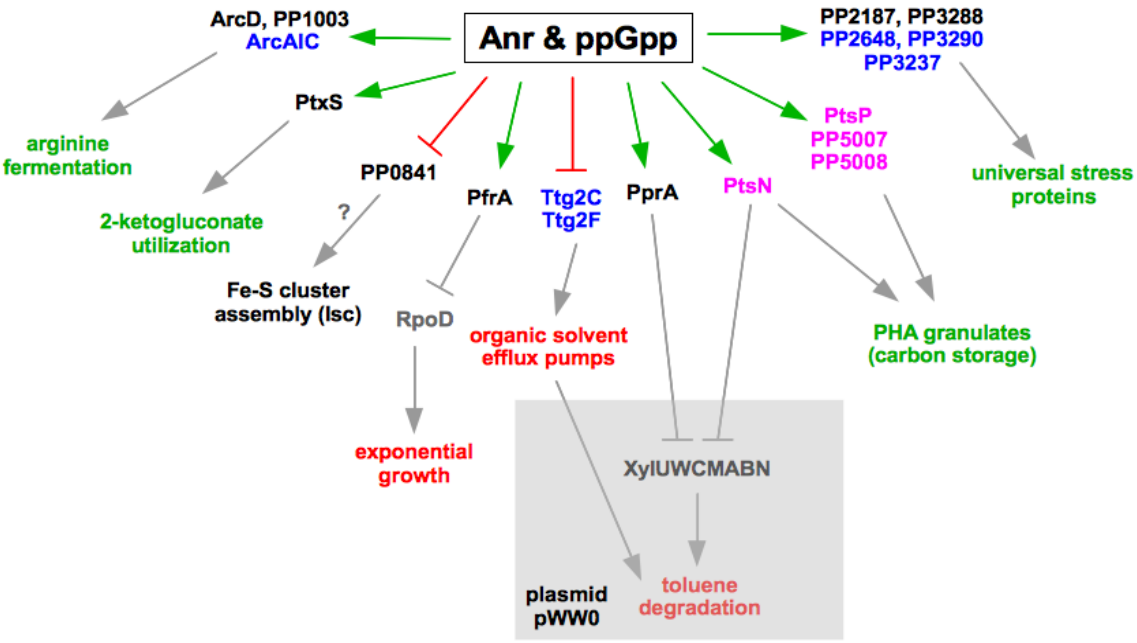

Fig. 37: Proposed regulatory network of Anr- and/or ppGpp-dependent gene expression after 30 minutes of oxygen depletion of mid-exponential phase *Pseudomonas putida* KT2440. Deductions were made based on transcription analysis described in this and preceding chapters. Gene products depicted black were found regulated by both Anr and ppGpp, gene products depicted in blue only by Anr and gene products depicted in magenta only by ppGpp. Green lines represent a positive regulation in response to oxygen depletion, red lines a negative regulation in response to oxygen depletion and gray lines regulatory connections indicated in literature. Cellular processes depicted in green are likely to be positively affected in response to oxygen depletion, cellular processes depicted in red are likely to be negatively affected in response to oxygen depletion. Note that in contrast to its parental strain mt-2, *P. putida* KT2440 does not carry plasmid pWW0 required for toluene degradation (indicated by gray background). For details please refer to text.

3.2.3 Introduction of *Pseudomonas aeruginosa* operons encoding denitrification steps in *Pseudomonas putida*

In *P. aeruginosa* PAO1 genes essential for denitrification are located in three genomic clusters, the *narK$_1$K$_2$GHIJLX* operon required for nitrate reduction, the *nirSMCFDLGHJEN-norBCD* operon mediating reduction of nitrite and nitric oxide, and the *nosDFLRYZ* operon catalyzing the conversion of nitrous oxide to dinitrogen (1.2.1.1). Orthologs of genes within these respective clusters are not present in *P. putida* KT2440 or other obligate aerobic Pseudomonas, although these strains possess orthologs of the neighboring genes of *P. aeruginosa* denitrification clusters (*moaA1* and PA3881 flanking the *narK$_1$K$_2$GHIJLX* operon, PA0508 and PA0528 flanking the *nirSMCFDLGHJEN-norBCD* operon, PA3389 and *fpr* flanking the *nosDFLRYZ* operon). This genomic organization suggests at the branching point of obligate aerobic and facultative anaerobic

Pseudomonads, the respective operons encoding denitrification steps were completely lost from the genome of ancestors of obligate aerobic strains like *P. putida*.
In order to gain a better understanding of the anaerobic metabolism in Pseudomonads, *P. aeruginosa* denitrification gene clusters were introduced to *P. putida* KT2440 and strains characterized regarding their ability of anaerobic growth or survival.

3.2.3.1 Isolation of *Pseudomonas aeruginosa* genes encoding dissimilatory nitrite and nitric oxide reductases

A *P. aeruginosa* ATCC17933 cosmid library (Schobert & Görisch, 1999) was used to gain gene clusters encoding reductases of the *P. aeruginosa* denitrification pathway. Cosmid pNQ07 encoding the *narK$_1$K$_2$GHIJLX* operon, was isolated by complementation of the anaerobic growth defect of a PAO1 Δ*narL* mutant strain in previous work (Quäck, 2005). In this study gene clusters encoding the *P. aeruginosa* ATCC17933 nitrite, nitric oxide and nitrous oxide reductases should be isolated.
The *P. aeruginosa nirSMCFDLGHJEN* operon encoding the dissimilatory nitrite reductase, is located adjacent to the *norBCD* operon encoding the nitric oxide reductase, in an approximately 15 kb gene cluster. Given an average insert size of the ATCC17933 cosmid library of 25 kb it is likely that a cosmid carrying both *nirSMCFDLGHJEN* and *norBCD* can be obtained.
In order to isolate the *nirSMCFDLGHJEN* and *norBCD* gene cluster it was attempted to complement the anaerobic growth deficiencies of several *Pseudomonas* strains. *P. putida* KT2440 as well as *P. aeruginosa* PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains (Liberati *et al.*, 2006), none of which are able to grow anaerobically in the presence of nitrite, served as recipient strains in separate screening approaches (2.8). The ATCC17933 cosmid library was introduced to the respective recipient strain via diparental mating (2.6.10) under aerobic conditions. In a next step strains were incubated anaerobically in the presence of nitrite in order to select for complemented mutants able to grow via the two-step reduction of nitrite to nitrous oxide.
No anaerobic growth on nitrite was observed when when *P. putida* KT2440 was used as a recipient strain. Meanwhile, complemented mutants of the PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains could be isolated in this experimental setup. Cosmids were isolated (2.6.9) and sequencing analysis (2.6.3) was carried out to determine the flanking regions of the inserted genomic DNA. Surprisingly, cosmids isolated via complementation of anaerobic growth of *P. aeruginosa* PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains did not contain the entire *nirSMCFDLGHJEN* operon.

During library screening with PA14 *nirS*::MrT7 transposon insertion mutant strain as recipient, cosmid pAS29a was isolated, containing a 26 kb insert covering the *P. aeruginosa* ATCC17933 genomic region from position 580917 (PA0520) to 606372 (PA0548). When PA14 *norB*::MrT7 transposon insertion mutant strain was used as recipient, pAS29a was also isolated, as well as a second cosmid, pAS29c, containing a 26 kb insert covering ATCC17933 genomic region 576764 (PA0516) to 603689 (PA0545).
At first glance, it seems puzzling no cosmid covering the entire *nirSMCFDLGHJEN* operon was isolated although nitrite served as electron acceptor in this experiment. However, both pAS29a and pAS29e carry the *norBCD* gene cluster. A possible explanation might be the necessity of bacteria to reduce nitric oxide, which has several undesirable effects on the cell. Nitric oxide is generated in small amounts by non-enzymatic reduction of nitrite, a process accelerated during acidic conditions (Zweier *et al.*, 1999). In biological systems nitric oxide readily reacts with metals within macromolecules, for instance in heme proteins such as guanylate cyclase or cytochromes (Wink & Mitchell, 1998). Indirect nitric oxide induced damaging effects are derived from the reaction of nitric oxide with either superoxide or oxygen, yielding reactive nitrogen oxide species (Wink & Mitchell, 1998). A third effect lies within the ability of small amounts of nitric oxide to act as a cellular signaling molecule (Hutchings *et al*., 2000; Van Alst *et al.*, 2007).
Therefore, it is possible only strains capable of reducing the accumulating nitric oxide are able to survive on nitrite. In case of PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains only complemented mutants carrying a cosmid containing *norBCD* would be able to survive on nitrite as well as grow in the presence of tetracycline due to the cosmid's resistance cassette. Several attempts to isolate the entire *nirSMCFDLGHJEN* operon by cosmid library screening with PA14 *nirS*::MrT7 and *norB*::MrT7 transposon insertion mutant strains as recipients were unsuccessful.
The next goal was to isolate cosmids carrying the 8 kb *nosDFLRYZ* operon encoding *P. aeruginosa* nitrous oxide reductase. It was shown for facultative anaerobic bacterium *P. stutzeri* that reduction of nitrous oxide to molecular nitrogen provides sufficient energy to promote anaerobic growth (Vollack & Zumft, 2001). Electron acceptor nitrous oxide as was generated via a PA14 *nosZ*::MrT7 transposon insertion mutant strain growing on nitrate, incubated in close proximity to the cosmid library screening setup (2.8). The lack of nitrous reductase activity is of no consequence for anaerobic growth of PA14 *nosZ*::MrT7 transposon insertion mutant strain, as nitrous oxide has no toxic or inhibitory effect on *P. aeruginosa* (Bryan *et al.*, 1985). *P. putida* KT2440 served as recipient strain in the cosmid library screening and should only grow in the presence of nitrous oxide when carrying a cosmid containing the *nosDFLRYZ* gene cluster.
No anaerobic growth of KT2440 was detectable after one week of incubation but several

colonies apparently survived the oxygen-depleted state and resumed growth as plates were reintroduced to an oxygenated environment. Cosmids were isolated (2.6.9) and further characterized by sequencing (2.6.3). Surprisingly, sequencing analysis revealed isolated cosmid pAS36 does not contain the *P. aeruginosa nosDFLRYZ* operon, although screening conditions were chosen to favor anaerobic growth or survival via nitrous oxide reduction. However, pAS36 covers the genomic region from position 565065 to 590708, containing the *nirSMCFDLGHJEN* and *norBCD* gene clusters (Fig. 38).

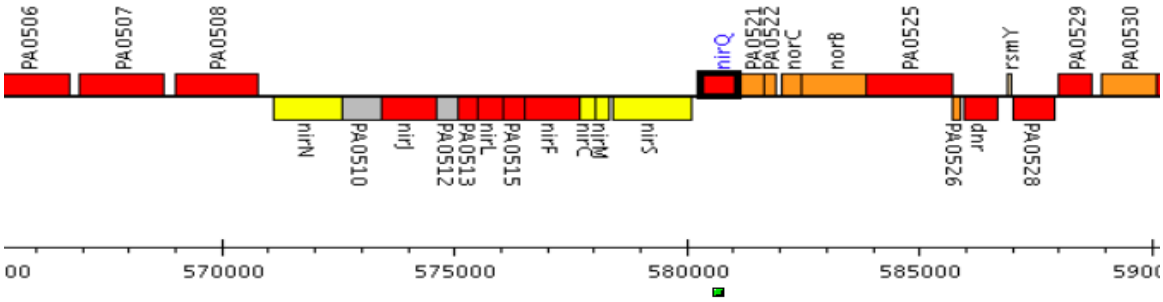

Fig. 38: Genomic organization of pAS36 covering the *Pseudomonas aeruginosa* PAO1 genome from position 565065 to 590708 including *nirSMCFDLGHJEN* and *norBCD* gene clusters, encoding nitrite and nitric oxide reductase, respectively, according to "*Pseudomonas* Genome Database". Cosmid library screening was performed as described elsewhere (2.8), obtained cosmid pAS36 was isolated (2.6.9), analyzed by restriction analysis (2.6.5) and sequencing (2.6.3). pAS36 contains a 25 kb insert, which covers functional genes PA0507 to PA0529.

Recipient strain *P. putida* KT2440 was offered no electron acceptor but nitrous oxide during the cosmid library screening, yet the isolated cosmid carries genes required for nitrite and nitric oxide reduction. The screening approach was carried out in complex LB medium which was shown to contain 5.5 µM nitrite (Van Alst *et al.*, 2009). Possibly, these small traces of nitrite were chemically reduced to nitric oxide (Zweier *et al.*, 1999). As isolated cosmid pAS36 contains the *norBCD* gene cluster, it is likely the presence of nitric oxide reductase allowed anaerobic survival of KT2440.

Goal of the cosmid library screening was to isolate *P. aeruginosa* PAO1 genomic region encoding steps of the denitrification pathway. The fact that all cosmids (pAS29a, pAS29e, pAS36) isolated in several experimental setups with various recipient strains carry the *norBCD* operon, emphasizes the importance of bacteria to dispose nitric oxide arising either enzymatically or non-enzymatically.

3.2.3.2 Anaerobic survival of *Pseudomonas putida* via nitrate reduction

Conversion of nitrate to nitrite is the highly energetic first reduction step of the *P. aeruginosa* denitrification chain. In fact, reduction of nitrate generates sufficient energy

for anaerobic growth of PAO1 Δ*nirS* mutant strains lacking genes encoding the subsequent reduction of nitrite (Barraud *et al*., 2006). Genes encoding catalytic and regulatory compounds necessary for nitrate reduction are located in the approximately 13 kb *narK₁K₂GHIJLX* operon. pLAFR3-derived cosmid pNQ07 containing *narK₁K₂GHIJLX* was previously isolated by complementation of the anaerobic growth defect of a PAO1 Δ*narL* mutant strain (Quäck, 2005).

In this study it was investigated if transfer of the *P. aeruginosa narK₁K₂GHIJLX* operon enables *P. putida* KT2440 anaerobic growth on nitrate. Cosmids pNQ07 and insert-free pLAFR3, respectively, were introduced to KT2440 by diparental mating (2.6.10), generating strains from now on referred to as KT2440-NAR and KT2440-pLAFR3. In a first step QRT-PCR (2.6.13) with a probe against *P. aeruginosa narG,* encoding part of the dissimilatory nitrate reductase, was carried out to determine if the *narK₁K₂GHIJLX* operon is transcribed in *P. putida*. KT2440-NAR was grown to mid-exponential phase with nitrate and shifted to anaerobic conditions, KT2440-pLAFR3 served as reference strain. Fold inductions of *narG* expression determined by QRT-PCR 0 and 3 h after the anaerobic shift are shown in Fig. 39.

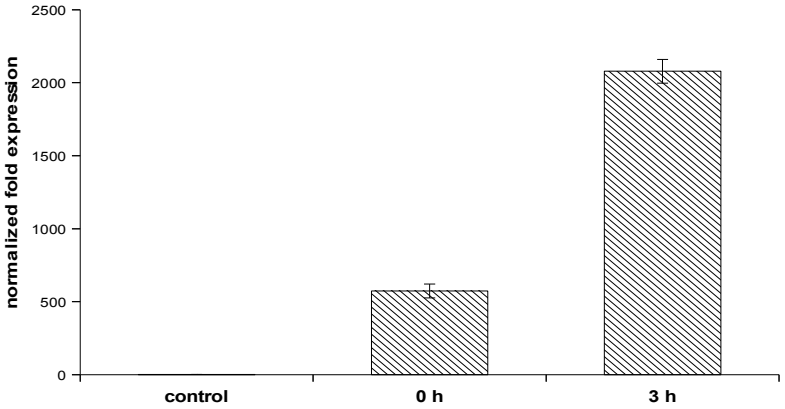

Fig. 39: Induction of *narG* expression in *Pseudomonas putida* KT2440-NAR, carrying the *Pseudomomas aeruginosa* ATCC17933 genes *narK₁K₂GHIJLX,* encoding for dissimilatory nitrate reductase, during anaerobic conditions with nitrate. After being grown aerobically to an OD_{578} of 0.5 in M9 supplemented with 50 mM KNO_3, strains KT2440-NAR and KT2440-pLAFR3 were incubated anaerobically in sealed serum flasks. At time points indicated, cells were harvested, total RNA was prepared (2.6.11) and transcribed into cDNA , which served as template for QRT-PCR (2.6.13). Gene expression was normalized with KT2440 *acpP* and PP5229 as reference genes, expression profile of KT2440-pLAFR3 prior to the anaerobic shift served as a control. *narG* transcript levels of KT2440-NAR were determined prior to (0 h) and after (3 h) the anaerobic shift. Error bars indicate standard error of the mean (SEM).

As seen in Fig. 39, QRT-PCR revealed that *narG* expression is induced 575-fold prior to the anaerobic shift in KT2440-NAR compared to control strain. After three hours of oxygen

depletion, *narG* expression levels are 2050-fold increased compared to the control, equating a 4-fold increase in *narG* expression with regard to values prior to the anaerobic shift.

In *P. aeruginosa* expression of *narGHI*, encoding the dissimilatory nitrate reductase, and *narK$_1$K$_2$,* encoding the cellular nitrate uptake system, are regulated by an interplay of Anr, Dnr and NarXL (Schreiber *et al.*, 2007). Oxygen-sensing transcriptional regulator Anr induces expression of the PAO1 *narK$_1$K$_2$GHIJLX* operon in response to oxygen limitation. It was shown for *P. aeruginosa* that several genes of the Anr regulon are induced even during mid-logarithmic aerobic growth due to microaerobic conditions induced by high cell densities and oxygen consumption (Trunk, 2005), which likely accounts for a basic induction of *narG* observed prior to the anaerobic shift. Two-component system NarXL, itself induced via Anr, senses nitrate availability and positively affects *narK$_1$K$_2$GHIJLX* expression. As nitrate was present in the medium this regulatory cascade might also be active prior to anaerobiosis. Nitric oxide sensor Dnr also participates in regulation of *narK$_1$K$_2$GHIJLX* transcription. However, as KT2440-NAR does not encode the *dnr* gene, Dnr-mediated regulation of *narK$_1$K$_2$GHIJLX* is of no consequence in KT2440-NAR.

In a next step it was determined if transcription of the *narK$_1$K$_2$GHIJLX* operon in KT2440, indicated by *narG* expression levels determined by QRT-PCR, also results in the formation of a functional dissimilatory nitrate reductase. For that purpose nitrate and nitrite consumption of KT2440-NAR and KT2440-pLAFR3, respectively, were measured in anaerobic shift experiments (experiments carried out by Özde Ütkür, Technische Universität Dortmund) (data not shown). No nitrate consumption was detected for KT2440-pLAFR3 over the course of six days during which the nitrate concentration in the medium remained constant at 20 mM and consequently, no accumulation of nitrite was observed. Meanwhile, the presence of *P. aeruginosa narK$_1$K$_2$GHIJLX* operon in strain KT2440-NAR an initial concentration of 20 mM nitrate decreased over time and eventually reached 1 mM after six days of incubation. A reverse pattern was observed for the concentration of nitrite, which was initially undetectable in the culture but increased to a final concentration of 18 mM after six days of incubation. These results strongly suggest ATCC17933 dissimilatory nitrate reductase is functional in *P. putida* KT2440.

As the *P. aeruginosa* dissimilatory nitrate reductase is apparently active in *P. putida* it was investigated if KT2440-NAR is able to grow anaerobically on nitrate. For this purpose cells were grown in a colony biofilm model on membrane filters (2.5.2), transferred daily on fresh medium to avoid inhibitory effects of accumulating nitrite. Although no anaerobic growth was detectable (data not shown) KT2440-NAR showed prolonged survival compared to KT2440-pLAFR3. This effect was further investigated in anaerobic shift experiments in which both strains were grown aerobically to mid-logarithmic phase with

nitrate, transferred into oxygen-impermeable sealed serum flasks and anaerobic survival was monitored (Fig. 40).

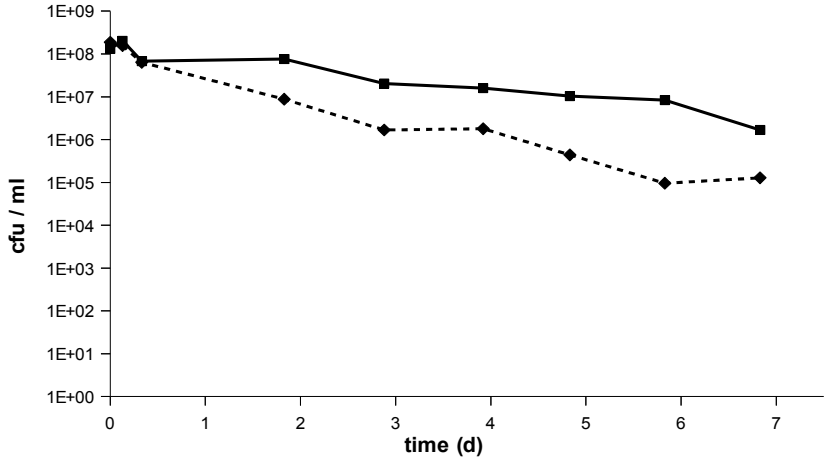

Fig. 40: Anaerobic planktonic survival of *Pseudomonas putida* KT2440-NAR, expressing *Pseudomonas aeruginosa* ATCC17933 genes *narK$_1$K$_2$GHIJLX,* encoding for dissimilatory nitrate reductase, with nitrate. Strains KT2440-NAR (solid line, squares) and KT2440-pLAFR3 (dotted line, diamonds) were grown aerobically to an OD_{578} of 0.5 in M9 supplemented with 50 mM KNO_3 and transferred to sealed serum flasks. Colony forming units (cfu/ml) were determined by plating (2.5.4) at time points indicated.

As seen in Fig. 40, the presence of *P. aeruginosa* dissimilatory nitrate reductase enhances anaerobic survival of *P. putida* KT2440-NAR in the presence of nitrate. After two days of incubation the number of colony forming units of KT2440-pLAFR3 were 10-fold reduced compared to KT2440-NAR. Up to six days the number of colony forming units of KT2440-NAR stagnates at approximately 1 x 10^7 cfu/ml, whereas those of KT2440-pLAFR3 are reduced to approximately 1 x 10^5 cfu/ml. No increased survival of KT2440-NAR was observed when cultures were not supplemented with nitrate (data not shown).
These results suggest that although *P. aeruginosa* nitrate reductase does not promote anaerobic growth of *P. putida* KT2440, it mediates increased anaerobic survival of the bacterium. However, KT2440 apparently lacks an important factor which allows energy obtained from nitrate reduction to be transmitted into active anaerobic growth.

3.2.3.3 Anaerobic survival of *Pseudomons putida* via nitrite and nitric oxide reduction

P. aeruginosa gene clusters *nirSMCFDLGHJEN* and *norBCD,* encoding dissimilatory nitrate and nitric oxide reductases, were isolated by cosmid library screening (3.2.3.1). It was demonstrated previously that *P. stutzeri nosZ* and *nosR* transposon insertion mutant strains are able to growth anaerobically on nitrite (Cuypers *et al.*, 1992), suggesting the

two-step reduction of nitrite to nitrous oxide promotes sufficient energy for anaerobic growth. In a next step it was investigated if nitrite and nitric oxide reductase activities promote anaerobic growth of *P. putida* KT2440 on nitrite. Cosmids pAS36 and insert-free pLAFR3, respectively, were introduced to KT2440 by diparental mating (2.6.10), generating strains from now on referred to as KT2440-NIR-NOR and KT2440-pLAFR3.
In a first step QRT-PCR (2.6.13) with probes against PAO1 *nirS* and *norB* was carried out to determine if *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons are induced in *P. putida* in response to anaerobic conditions with nitrite. *P. putida* KT2440-NIR-NOR was grown aerobically to mid-exponential phase and shifted to anaerobic conditions with nitrite, KT2440-pLAFR3 served as a reference strain. Fold inductions of *nirS* and *norB* expression were determined by QRT-PCR after 0, 1 and 3 hours of oxygen depletion are shown in Fig. 41.

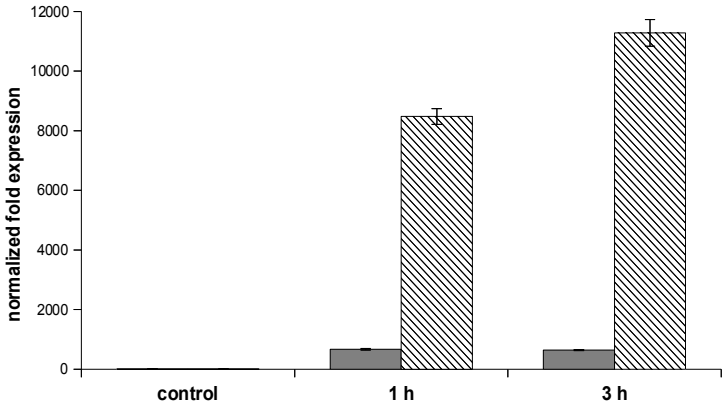

Fig. 41: Induction of (gray bars) *nirS* and (hatched bars) *norB* expression in *Pseudomonas putida* KT2440-NIR-NOR, containing *Pseudomonas aeruginosa* ATCC17933 genes *nirSMCFDLGHJEN* and *norBCD,* encoding the dissimilatory nitrite and nitric oxide reductase, respectively, after transition to anaerobic conditions with nitrite. After being grown aerobically to an OD_{578} of 0.5 in M9, strains KT2440-NIR-NOR and KT2440-pLAFR3 were incubated anaerobically in sealed serum flasks for time periods indicated. 5 mM $NaNO_2$ were added at the time point of the anaerobic shift. At time points indicated, cells were harvested, total RNA was prepared (2.6.11) and transcribed into cDNA, which served as template for QRT-PCR (2.6.13). Gene expression was normalized with KT2440 *acpP* and PP5229 as reference genes, expression profile of KT2440-pLAFR3 prior to the anaerobic shift served as a control. *nirS* and *norB* transcript levels of KT2440-NIR-NOR were determined after the anaerobic shift (1 h and 3 h). Error bars indicate standard error of the mean (SEM).

As seen in Fig. 41, oxygen depletion together with the addition of nitrite resulted in a strong increase of *norB* expression in strain KT2440-NIR-NOR, up to 8500-fold induction after one hour of anaerobic incubation compared to KT2440-pLAFR3 prior to the anaerobic shift. Meanwhile, expression of dissimilatory nitrite reductase encoding *nirS*, was induced 650-fold compared to the control after one hour. Whereas *norB* expression was further increased after three hours of anaerobiosis (11000-fold), *nirS* transcript levels

remained constant (650-fold).

In *P. aeruginosa* expression of *nirSMCFDLGHJEN* and *norBCD* operons is under control of transcriptional regulator Dnr, whose expression is regulated by oxygen-sensor Anr (Arai *et al.*, 1997). Once induced upon oxygen limitation Dnr senses nitric oxide (Arai *et al.,* 1995 a) and mediates gene expression accordingly. *P. aeruginosa dnr* is located downstream of the *norBCD* and also present on cosmid pAS36. Consequently, *P. putida* KT2440 is able to sense nitric oxide via Dnr, resulting in Dnr-dependent activation *nirSMCFDLGHJEN* and *norBCD* expression. Once reduction of nitrite to nitric oxide by dissimilatory nitrite reductase NirS begins, an increased amount of NorBC nitric oxide reductase is essential for a rapid detoxification of toxic nitric oxide, which explains the drastic increase of *norB* transcription in KT2440-NIR-NOR during anaerobic incubation.

In a next step it was determined if transcription of the *nirSMCFDLGHJEN* and *norBCD* operons in KT2440, indicated by *nirS* and *norB* expression levels determined by QRT-PCR, results in the formation of a functional dissimilatory nitrite reductase. For that purpose nitrite consumption rates of KT2440-NIR-NOR and KT2440-pLAFR3, respectively, were measured in anaerobic shift experiments (experiments carried out by Özde Ütkür, Technische Universität Dortmund) (data not shown). No nitrite consumption was detected for KT2440-pLAFR3 over the course of six days during which nitrite concentrations in the medium remained constant at 15 mM. Meanwhile, in the presence of *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons in strain KT2440-NIR-NOR, an initial concentration of 15 mM nitrite decreased over time and eventually reached 0.5 mM after six days of incubation. These results suggest the *P. aeruginosa* nitrite reductase is indeed active in *P. putida* KT2440-NIR-NOR. Additionally, it gives indirect evidence that *P. aeruginosa* nitric oxide reductase is also active, as accumulation of nitric oxide would likely have lethal effects on the cells and thus abolish nitrite consumption.

In a next step it was investigated if reduction of nitrite and nitric oxide promotes *P. putida* anaerobic growth in liquid cultures supplemented with nitrite. As shown for *P. aeruginosa* dissimilatory nitrate reductase (3.2.3.2), introduction nitrite and nitric oxide reductase also did not promote anaerobic growth of *P. putida* KT2440 (data not shown). To verify if nitrite and nitric oxide reductase might contribute to anaerobic survival, anaerobic shift experiments were carried out. For that purpose KT2440-NIR-NOR and KT2440-pLAFR3 were grown to mid-exponential phase and subsequently transferred to oxygen-impermeable sealed serum flasks with nitrite. Anaerobic survival was monitored over the course of seven days (Fig. 42).

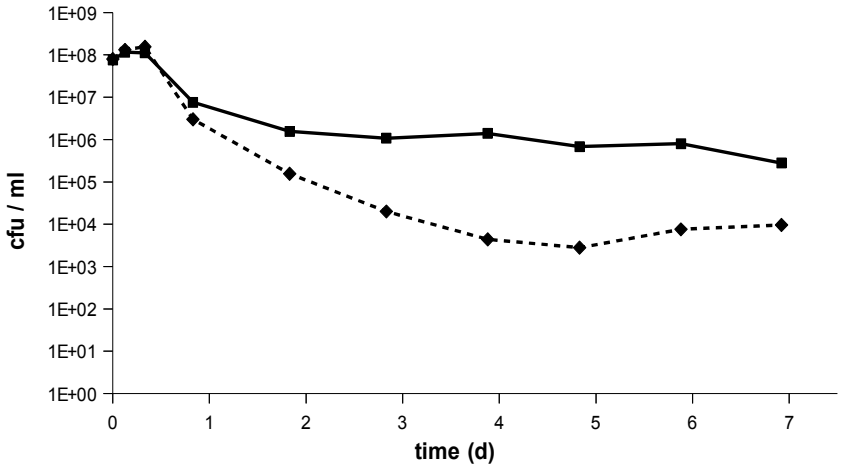

Fig. 42: Anaerobic planktonic survival of *Pseudomonas putida* KT2440-NIR-NOR, expressing *Pseudomonas aeruginosa* ATCC17933 genes *nirSMCFDLGHJEN* and *norBCD,* encoding for dissimilatory nitrite reductase and nitric oxide reductase, respectively, with nitrite. Strains KT2440-NIR-NOR (solid line, squares) and KT2440-pLAFR3 (dotted line, diamonds) were grown aerobically to an OD_{578} of 0.5 in M9 and transferred into sealed serum flasks. At time point of the anaerobic shift, 5 mM $NaNO_2$ was added to cultures. Colony forming units (cfu/ml) were determined by plating (2.5.4) at time points indicated.

As seen in Fig. 42, the presence of *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* gene clusters increases anaerobic survival of KT2440-NIR-NOR with nitrite. After one day of incubation viable cell numbers of KT2440-pLAFR3 are 50 % lower than those of KT2440-NIR-NOR. Increased survival rates of KT2440-NIR-NOR became more pronounced over the course of incubation. After five days the maximum difference in the number of colony forming units was visible, with approximately 7 x 10^5 cfu/ml detected for KT2440-NIR-NOR and approximately 3 x 10^3 cfu/ml detected for KT2440-pLAFR3. These results strongly suggest that reduction of nitrite to nitrous oxide contributes *P. putida* anaerobic survival, although growth was not promoted.

3.2.3.4 *In silico* identification of genes with potential roles in anaerobic metabolism of Pseudomonads

In order to identify genes other than the denitrification gene clusters, which might be essential for anaerobic growth of Pseudomonads, genomes of obligate aerobic and facultative anaerobic strains were compared *in silico*. Genome comparison was carried out with the "Comparative Genome Search" function provided by "*Pseudomonas* Genome Database" (2.10.4). This service was used to identify genes with a potential role during anaerobic growth, which should be present in the genomes of facultative anaerobic strains *P. aeruginosa* and *P. stutzeri* but absent the genomes of obligate aerobic strains *P. putida*,

P. fluorescens, P. mendocina, P. syringae and *P. entomophila*.

137 genes were annotated in the genomes of *P. aeruginosa* PAO1 and *P. stutzeri* A1501 but not in the genomes of the above mentioned obligate aerobic Pseudomonads. Of these genes 55 encode factors directly involved in denitrification steps, which were excluded from further analysis. According to "*Pseudomonas* Genome Database" the remaining 82 genes were categorized in four classes, with the function of class I genes being extensively characterized and class IV genes encoding hypothetical or conserved hypothetical products (details on classification criteria are described in 2.10.4). Based on this classification genes encoded solely by facultative anaerobic *Pseudomonas* strains were further examined, in particular 19 genes which were regarded as class I and class II genes.

Several class I genes were previously implicated in cellular processes important during anaerobic conditions. Among them was the PAO1 *nrdJab* two-gene cluster encoding a class two oxygen-independent ribonucleotide-diphosphate reductase required for the reduction of ribonucleotides to 2-deoxyribonucleotides in a radical-dependent mechanism (Torrents *et al.*, 2005; Jordan *et al.*, 1999). As an obligate aerobic strain *P. putida* possesses a class one oxygen-dependent ribonucleotide-diphosphate reductase but not an ortholog of *nrdJab.* In order to investigate if the absence of NrdJab accounts for the inability of KT2440-NAR and KT2440-NIR-NOR to grow anaerobically via dissimilatory nitrate as well as nitrite and nitrous oxide reduction, respectively (3.2.3.2 and 3.2.3.3), PAO1 *nrdJab* genes were introduced into these strains. However, the presence of *nrdJab* had no positive effect on anaerobic growth and survival of KT2440-NAR and KT2440-NIR-NOR (data not shown).

P. putida also lacks orthologs of genes *nrdDG* encoding a class three anaerobic ribonucleotide-diphosphate reductase. However, as no class three anaerobic ribonucleotide-diphosphate reductase enzyme activity could be detected for *P. aeruginosa* during oxygen-depleted conditions (Jordan *et al.*, 1999), it is unlikely NrdDG contribute to anaerobic growth in *P. aeruginosa*.

Other *in silico* identified genes which were categorized as class I genes include *P. aeruginosa pilY2* which is involved type IV pili biosynthesis. Type IV pili were shown to be required for anaerobic, but not aerobic, biofilm formation (Yoon *et al.*, 2002). No connection of other class I and class II genes to the anaerobic metabolism is obvious from literature. Interestingly, numerous class III genes encode a diverse array of transporters although it is unknown if one or more of these gene products is of importance for *P. aeruginosa* or *P. stutzeri* anaerobic growth.

Genes identified as present in facultative anaerobic but not obligate aerobic *Pseudomonas* strains by "Comparative Genome Search" with "*Pseudomonas* Genome

Database" are listed in Appendix I.

3.2.3.5 Gene expression profile of *Pseudomonas putida* during anaerobic nitrate reduction

Introduction of the *P. aeruginosa* dissimilatory nitrate reductase increases *P. putida* KT2440 anaerobic survival in the presence of nitrate (3.2.3.2). Transcriptome analysis was carried out in order to gain hints why the *narK$_1$K$_2$GHIJLX* operon promotes KT2440-NAR anaerobic survival but not growth. For that purpose KT2440-NAR and KT2440-pLAFR3 were grown aerobically to an OD_{578} of 0.5 in the presence of nitrate, transferred to oxygen-impermeable sealed serum flasks and incubated anaerobically. Cells for microarray analysis were harvested prior to the anaerobic shift and after 30 minutes of anaerobiosis. Total RNA was prepared (2.6.11), analyzed with Agilent 2100 Bioanalyzer and transcriptome analysis was carried out with Agilent gene expression oligo microarrays (2.9.2). Raw microarray data was preprocessed, fold change expression values calculated (2.9.2) and transcriptome profiles were analyzed according to the diagram depicted in Fig. 43.

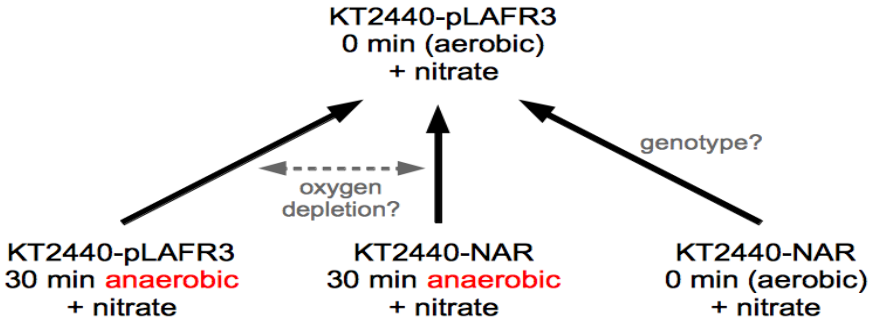

Fig. 43: Schematic overview of data analysis to determine effects of the *Pseudomonas aeruginosa* ATCC17933 *narK$_1$K$_2$GHIJLX* operon, encoding the dissimilatory nitrate reductase, on transcriptome profiles of *Pseudomonas putida* KT2440-NAR in response to oxygen depletion. Experimental procedures are described in text. Solid arrows indicate two-dye Agilent gene expression oligo microarrays carried out in triplicate, dotted arrows comparisons made for data analysis. Expression profiles of KT2440-pLAFR3 prior to anaerobic shift served as reference sample. To determine the transcriptional response of KT2440-NAR to the presence of *P. aeruginosa narK$_1$K$_2$GHIJLX* operon independently of oxygen depletion, expression profiles of KT2440-NAR and KT2440-pLAFR3, respectively, prior to anaerobic shift were compared. Transcriptome data obtained for KT2440-NAR and KT2440-pLAFR3, respectively, after 30 minutes of oxygen depletion were compared in order to unveil differences in gene regulation during anaerobic conditions.

As seen in Fig. 43, several transcriptome profiles were generated to determine gene expression of KT2440-NAR in response to oxygen-limiting conditions. Effects of the

P. aeruginosa *narK₁K₂GHIJLX* operon on expression profiles of KT2440-NAR independently of oxygen depletion were investigated by comparison of transcriptomes of KT2440-NAR and KT2440-pLAFR3, respectively, prior to the anaerobic shift. Expression profiles of KT2440-NAR after 30 minutes of oxygen depletion were compared to those of KT2440-pLAFR3 30 minutes after the anaerobic shift in order to investigate similarities and differences in transcriptional responses. Based on this analysis genes differentially regulated in KT2440-NAR after 30 minutes of oxygen depletion were identified. Appendix J lists all genes differentially regulated in KT2440-NAR after 30 minutes of anaerobic incubation compared to KT2440-pLAFR3 prior to the anaerobic shift and their respective fold changes. Additionally, identified genes were classified in those dependent on oxygen depletion and those constitutively expressed during the investigated conditions (Fig. 44).

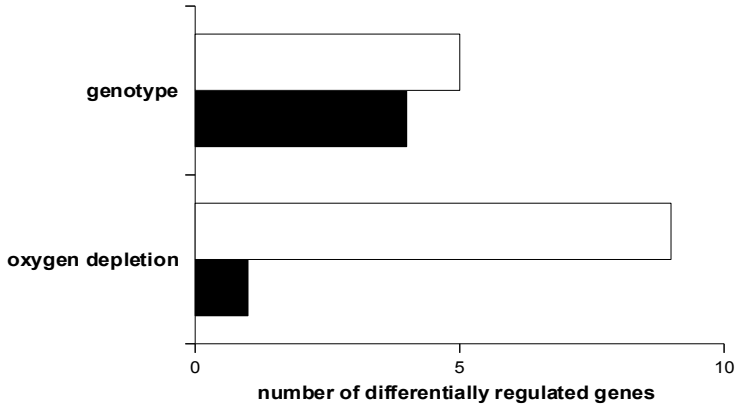

Fig. 44: Dependency of genes differentially expressed in *Pseudomonas putida* KT2440-NAR, containing the *Pseudomonas aeruginosa* ATCC17933 *narK₁K₂GHIJLX* operon, encoding the dissimilatory nitrate reductase, on genotype or oxygen depletion. Experimental procedures are described in text. Transcriptome profiles were analyzed according to the diagram depicted in Fig. 43. Fold change expression values for KT2440-NAR after 30 minutes of anaerobic incubation with nitrite were compared to expression profiles prior to the anaerobic shift. White bars indicate induced genes, black bars repressed genes. Note that differentially regulated genes of the *P. aeruginosa* *narK₁K₂GHIJLX* operon are not included here.

As seen in Fig. 44, 19 *P. putida* KT2440 genes are differentially regulated in KT2440-NAR after 30 minutes of oxygen depletion in the presence of nitrate. The largest number of genes is induced in KT2440-NAR only in response to oxygen depletion whereas only one gene is repressed upon the anaerobic shift. However, several genes are differentially expressed prior to the anaerobic shift, likely due to the fact that most *P. aeruginosa* nitrate reductase encoding genes are also induced prior to oxygen depletion (Appendix J).

In order to gain hints on the contribution of *P. aeruginosa* nitrate reductase to *P. putida* anaerobic survival (3.2.3.2), genes differentially regulated in KT2440-NAR were analyzed in detail. Noticeably, various genes of a gene cluster ranging from PP5389 to PP5401 are

upregulated in KT2440-NAR in comparison to KT2440-pLAFR3 prior to the anaerobic shift. More importantly, a strong increase in expression of most genes of this cluster was observed after 30 minutes of oxygen depletion. Expression of several of these genes was shown to be regulated by Anr as well as the stringent response after 30 minutes of oxygen depletion (Appendix G and H). Interestingly, PP5392 is an ortholog of *P. aeruginosa nirF* encoding a protein involved in heme d_1 biosynthesis (Kawasaki *et al.*, 1995). Other genes of this cluster encode mainly hypothetical proteins, although a few are proposed to encode proteins required for copper homeostasis (Cánovas *et al.*, 2003). For instance, gene products of PP5389 and PP5394 are proposed to mediate efflux of di- and monovalent cations, respectively. PP5393 shows a strong induction (108-fold) in KT2440-NAR 30 minutes after the anaerobic shift and shares similarities with *P. fluorescens cueZ*, which is expressed in response to elevated copper levels (Zhang & Rainey, 2008). Although *cueZ* itself encodes a copper chaperone protein which possibly delivers copper ions to copper-requiring cellular enzymes, is part of a regulon induced in order to export copper from the cell. An upregulation of gene cluster PP5389 to PP5401 in KT2440-NAR implicates an elevated intracellular level of copper in this strain. Despite of its importance as an enzymatic cofactor, an excess of copper is highly toxic to the bacterial cell. Enzymes which require copper are located extracytoplasmically and are found mainly in the periplasm or embedded in the cytoplasmic membrane (Osman & Cavet, 2008). Interestingly, copper was shown to be more toxic under anaerobic conditions likely resulting from a reduction of Cu^{2+} to Cu^{1+}, which can diffuse through the cytoplasmic membrane causing elevated intracellular copper levels (Outten *et al.*, 2001). As copper was shown to generate hydroxyl radicals via a Fenton-type reaction *in vitro* (Gunther *et al.*, 1995) it was proposed an increased oxidative stress is responsible for copper toxicity. However, although deletion of genes encoding copper export systems resulted in a 20-fold increase of intracellular copper concentrations and suppressed growth in *E. coli*, high amounts of copper did actually decrease hydrogen peroxide mediated DNA damage (Macomber *et al*., 2007). Although nucleic acids appear not to be affected by elevated copper concentrations, activities of several dehydratases containing FeS clusters, such as isopropylmalate isomerase, fumarase A and 6-phosphogluconate dehydratase, were compromised in *E. coli* mutant strains unable to export copper (Macomber & Imlay, 2009). In addition, copper was shown to damage the FeS cluster of fumarase A *in vitro*, implicating copper might also affect the FeS cluster of *P. aeruginosa* nitrate reductase in KT2440-NAR. As copper toxicity was reported to increase in the absence of oxygen, an observed increased transcription of genes potentially involved in mediating *P. putida* copper homeostasis upon oxygen depletion, is of great interest for further analysis.

Only a few genes were downregulated in KT2440-NAR, but not KT2440-pLAFR3 after

30 minutes of oxygen depletion. These include genes PP3781, PP3783 and PP3785 of an operon related to biosynthesis of lipodepsinonapeptide class phytotoxins like syringomycin and barbamide (Nelson *et al.*, 2002). Operon PP3781 to PP3790 was shown to be repressed via MvaT-like transcriptional regulators TurA (TOL upper operon repressor) and TurB in mid-exponential growth phase (Renzi *et al.*, 2010).

Gene PP1249 which is also downregulated in KT2440-NAR in response to oxygen depletion, was implicated to play a role in the uptake of L-hydroxyproline, a major constituent of collagen and plant cell wall proteins (Brouns *et al*., 2007), which *P. putida* converts to TCA cycle intermediate α-ketoglutarate. Expression of genes PP1249 as well as PP3781 and PP3783 was shown to be downregulated in KT2440 grown aerobically in the presence of 2,4,6-trinitrotoluene (TNT) (Fernandéz *et al*., 2009).

In conclusion, the transcriptomic response of KT2440-NAR suggests the strain might be unable to grow anaerobically via nitrate reduction due to effects caused by an intracellular accumulation of copper. The molecule might exert damage on FeS clusters and thus affecting the enzymatic activity of *P. aeruginosa* nitrate reductase. In addition, a few genes which appear to be involved in carbon compound catabolism were downregulated in KT2440-NAR in response to oxygen depletion, although the reason for this remains unknown.

3.2.3.6 Gene expression profile of *Pseudomonas putida* during anaerobic nitrite and nitric oxide reduction

Introduction of dissimilatory nitrite and nitric oxide reductases increases *P. putida* KT2440 anaerobic survival in the presence of nitrite (3.2.3.3). Transcriptome analysis was carried out in order to determine why *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons promote KT2440-NIR-NOR anaerobic survival but not growth. For that purpose KT2440-NIR-NOR and KT2440-pLAFR3 were grown aerobically to an OD_{578} of 0.5, transferred to oxygen-impermeable sealed serum flasks and incubated anaerobically with nitrite. Additional cultures were grown to an OD_{578} of 0.5 and incubated aerobically with nitrite in order to determine the effects of nitrite but not oxygen depletion on gene expression. Cells for microarray analysis were harvested prior to the anaerobic shift and after 30 minutes of anaerobiosis or addition of nitrite. Total RNA was prepared (2.6.11), analyzed with Agilent 2100 Bioanalyzer and transcriptome analysis was carried out with Agilent gene expression oligo microarrays (2.9.2). Raw microarray data was preprocessed, fold change expression values calculated (2.9.2) and transcriptome profiles were analyzed according to the diagram depicted in Fig. 45.

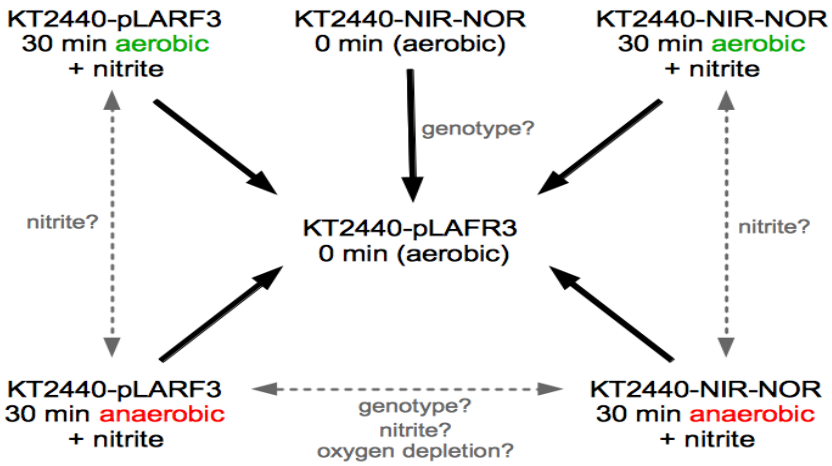

Fig. 45: Schematic overview of data analysis to determine effects of *Pseudomonas aeruginosa* ATCC17933 *nirSMCFDLGHJEN* and *norBCD* operons, encoding dissimilatory nitrite reductase and nitric oxide reductase, respectively, on transcriptome profiles of *Pseudomonas putida* KT2440-NIR-NOR in response to oxygen depletion. Experimental procedures are described in text. Solid arrows indicate two-dye Agilent gene expression oligo microarrays carried out in triplicate, dotted arrows comparisons made for data analysis. Expression profiles of KT2440-pLAFR3 prior to anaerobic shift served as reference sample. Comparison of transcriptome profiles of KT2440-NIR-NOR and KT2440-pLAFR3, respectively, after 30 minutes of anaerobic incubation with nitrite were used to determine genotype-specific expression in response to oxygen depletion and nitrite addition. To determine the transcriptional response of KT2440-NIR-NOR to the presence of *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons independently of oxygen depletion, expression profiles of KT2440-NAR and KT2440-pLAFR3, respectively, prior to anaerobic shift were compared. Additionally, expression profiles obtained for both KT2440-NIR-NOR and KT2440-pLAFR3 after 30 minutes of aerobic incubation with nitrite were carried out to determine nitrite-dependent gene regulation independently of anaerobiosis.

Appendix K lists all *P. putida* KT2440 genes differentially regulated in KT2440-NIR-NOR after 30 minutes of anaerobic incubation compared to KT2440-pLAFR3 prior to the anaerobic shift and their respective fold changes. In order to distinguish effects of genotype, nitrite and oxygen depletion on expression profiles of KT2440-NIR-NOR, obtained transcriptome profiles were cross-examined (Fig. 45). To determine genotype-dependent effects due to the presence of *P. aeruginosa nirSMCFDLGHJEN-norBCD* clusters independently of oxygen depletion expression profiles of KT2440-NIR-NOR and KT2440-pLAFR3, respectively, prior to the anaerobic shift were compared. As nitrite was added to the culture upon the anaerobic shift, nitrite-related effects on gene expression independently of anaerobiosis were analyzed with expression profiles obtained for both KT2440-NIR-NOR and KT2440-pLAFR3 after 30 minutes of aerobic incubation with nitrite.

Based on this analysis it was determined if genes differentially expressed in KT2440-NIR-

NOR after 30 minutes of anaerobic incubation with nitrite, were regulated in response to the presence of nitrite, a depletion of oxygen or if they are constitutively expressed during the investigated conditions (Fig. 46).

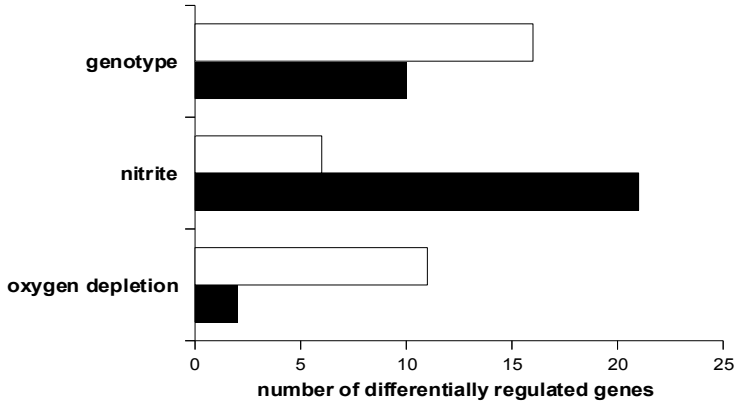

Fig. 46: Dependency of KT2440 genes differentially expressed in *Pseudomonas putida* KT2440-NIR-NOR, containing the *Pseudomonas aeruginosa* ATCC17933 *nirSMCFDLGHJEN-norBCD* operon, on genotype, the presence of nitrite and oxygen depletion. Experimental procedures are described in text. Transcriptome profiles were analyzed according to the diagram depicted in Fig. 45. Fold change expression values obtained for KT2440-NIR-NOR after 30 minutes of anaerobic incubation with nitrite were compared to expression profiles prior to the anaerobic shift, as well as to expression profiles after 30 minutes of aerobic incubation with nitrite. White bars indicate induced genes, black bars repressed genes. Note that differentially regulated genes of the *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons are not included here.

As seen in Fig. 46, after 30 minutes of oxygen depletion in the presence of nitrite 66 *P. putida* KT2440 genes are differentially regulated in KT2440-NIR-NOR. A large number of these genes is already differentially expressed prior to the anaerobic shift, likely due to the fact that several genes of the *P. aeruginosa nirSMCFDLGHJEN* and *norBCD* operons are also induced prior to oxygen depletion (Appendix K). Only a small number of genes upregulated 30 minutes after the anaerobic shift is induced in response to nitrite, whereas several genes were induced due to oxygen depletion. In contrast, among the genes downregulated 30 minutes after the anaerobic shift a large number of genes is repressed due to the addition of nitrite.

In order to gain insights on the contribution of *P. aeruginosa* nitrite and nitric oxide reductases to *P. putida* anaerobic survival (3.2.3.3) expression profiles of KT2440-NIR-NOR were analyzed in detail. As observed in similar transcriptome analysis with KT2440-NAR encoding the *P. aeruginosa* dissimilatory nitrate reductase (3.2.3.5), various genes of a cluster ranging from PP5389 to PP5401 are highly upregulated in KT2440-NIR-NOR in response to oxygen depletion. As discussed before (3.2.3.5), several of these genes were implicated to contribute to *P. putida* copper homeostasis (Cánovas *et al.*, 2003) and it was

proposed copper might damage the FeS cluster of *P. aeruginosa* nitrate reductase in KT2440-NAR. However, neither *P. aeruginosa* nitrite or nitric oxide reductase possess a FeS cluster suggesting a potential accumulation of copper causes other effects which prevent anaerobic growth of KT2440-NIR-NOR.

Additionally, several genes encoding ribosomal proteins or ribosome binding proteins were induced in KT2440-NIR-NOR, as well as genes PP2358 to PP2360, implicated to play a role in type I pilus formation (Kivistik *et al*., 2006). Lastly, two transcriptional regulators with unknown functions, PP4424 and PP3684.1, were upregulated in KT2440-NIR-NOR.

Among the genes downregulated in KT2440-NIR-NOR, which were at large regulated due to the addition of nitrite and independently of oxygen availability, are several whose products contribute to the bacterial stress response. For instance, expression of genes *groES* and *groEL* encoding molecular chaperones which protect newly synthesized or stress-denatured polypeptides from misfolding or aggregation (Frydman, 2001), is repressed in KT2440-NIR-NOR. Expression of *sodA,* encoding a superoxide dismutase which protects cells from oxidative stress by conversion of superoxide to hydrogen peroxide and molecular oxygen, is also downregulated in KT2440-NIR-NOR.

As observed for KT2440-NAR several genes of the TurA- and TurB-dependent operon encoded by PP3781 to PP3790 (Renzi *et al.*, 2010) related to lipodepsinonapeptide class phytotoxin biosynthesis, were downregulated in KT2440-NIR-NOR in response to oxygen depletion as well as nitrite. Additionally, several genes of the downstream operon, PP3775 to PP3780, were repressed in KT2440-NIR-NOR after 30 minutes of oxygen depletion. Genes of this operon were also shown to be downregulated via TurA, but not TurB, during *P. putida* mid-exponential growth (Renzi *et al.*, 2010) and are proposed to be involved in amino acid biosynthesis and metabolism.

A number of genes of a pyoverdine biosynthetic cluster ranging from approximately PP4213 to PP4246 were repressed in KT2440-NIR-NOR upon addition of nitrite, independently of oxygen availability. Pyoverdines are Fe^{3+} chelators produced during aerobic conditions in response to iron limitation and also function as signaling molecules, for instance in virulence factor production (Ravel & Cornelis, 2003). In agreement with a downregulation of a pyoverdine biosynthetic gene cluster, PP0947 encoding a zinc/iron permease was repressed in KT2440-NIR-NOR. Also, PP3808 encoding an MbtH-like protein, which is an integral component of bacterial nonribosomal peptide synthetases (Felnagle *et al*., 2010) mediating pyoverdine biosynthesis (Mossialos *et al.*, 2002), was downregulated in KT2440-NIR-NOR in response to the addition of nitrite. Superoxide dismutase encoding *sodA* was upregulated in response to iron starvation in *Aspergillus* (Oberegger *et al.*, 2000) whereas the gene is repressed in KT2440-NIR-NOR.

These findings implicate a decreased iron uptake in *P. putida* expressing the

P. aeruginosa nirSMCFDLGHJEN and *norBCD* operons. Given that the production of nitrite and nitric oxide reductase would result in an increased iron requirement, as iron is an important cofactor of these enzymes, this seems paradox. However, a possible copper stress indicated by the upregulation of genes PP5389 to PP5401 might interfere with the metabolism of other heavy metals, such as iron.

In conclusion, the transcriptional response of KT2440-NIR-NOR upon oxygen depletion in the presence of nitrite resembles that determined for KT2440-NAR in the presence of nitrate (3.2.3.5). These findings suggest a similar cause for the inability of *P. aeruginosa* nitrate as well as nitrite and nitric oxide reductases to promote anaerobic growth of *P. putida* KT2440. Transcriptome data implicates copper-induced stress might play a role in both cases. Possibly, *P. putida* lacks an additional system required for copper detoxification present in facultative anaerobic Pseudomonads. Comparative genome analysis carried out in order to determine genes which might be important for anaerobic growth (3.2.3.4) revealed a *P. stutzeri* A1501 gene encoding a putative copper export protein (PST0944). An ortholog of PST0944 is present in *P. aeruginosa* PAO1 (PA5250, encoding a conserved hypothetical protein) but not in obligate aerobic *Pseudomonas* strains.

4. Summary and Outlook

4.1 Summary

In this study adaptation of two related bacteria, *Pseudomonas aeruginosa* and *Pseudomonas putida*, to changes in nutrient and oxygen availability was investigated. The stringent response, which is activated by a number of stress conditions and controlled by an interplay of proteins RelA, SpoT and DksA, as well as the oxygen-sensing transcriptional regulator Anr were the main focus.

Four *P. aeruginosa* mutant strains affected in their ability to carry out the stringent response were characterized in detail. It was shown that *P. aeruginosa* PAO1 strains unable to synthesize either RelA and SpoT or DksA have a severe growth defect in the absence of oxygen, particularly during colony biofilm growth. This defect was to some extent abolished by rendering the strains unable to produce nitric oxide via denitrification, suggesting nitric oxide mediates the anaerobic colony biofilm growth defect of *P. aeruginosa* strains affected in the stringent response. In order to determine the roles of ppGpp and DksA in gene regulation under anaerobic conditions a comprehensive transcriptome analysis using the respective mutant strains was carried out. It was found that ppGpp and DksA are involved in regulation of cellular processes contributing to *P. aeruginosa* pathogenesis, like alginate biosynthesis, type VI secretion and biofilm formation. Transcriptome data did not directly indicate an accumulation of nitric oxide via denitrification in strains unable to carry out the stringent response, however, it indicated a deregulation of several genes encoding proteins involved in denitrification.

In the second part of this study adaptation of the obligate aerobic bacterium *P. putida* KT2440 to decreased oxygen availability was investigated. Transcriptome analysis was carried out in order to determine the initial response of KT2440 to oxygen depletion, mediated by the stringent response and the global, oxygen-sensing transcriptional regulator Anr. Additionally, *in silico* predicted Anr binding sites of KT2440 and *P. aeruginosa* PAO1 were compared in order to investigate differences of Anr-mediated gene expression in obligate aerobic and facultative anaerobic Pseudomonads. In a next step gene clusters encoding the nitrate reductase as well as the nitrite and nitric oxide reductases of the *P. aeruginosa* denitrification system were characterized to elucidate their ability to promote *P. putida* anaerobic growth. Introduction of PAO1 nitrate or nitrite and nitric oxide reductases did not enable KT2440 anaerobic growth but mediated a prolonged anaerobic survival. Transcriptome analysis carried out in order to investigate the underlying mechanism revealed that expression of the denitrification genes might increase copper stress in *P. putida*.

4.2 Outlook

A contribution of nitric oxide to the growth defect of *P. aeruginosa* mutant strains unable to carry out the stringent response during anaerobic conditions remains to be evaluated, for instance by incubation of these strains with nitric oxide scavenging reagents or direct measurement of nitrite and nitric oxide reductase activities. Several genes which were identified as regulated by the stringent response and previously implicated in *P. aeruginosa* biofilm growth, also need to be investigated regarding their potential role during anaerobic colony biofilm growth. This could be achieved by generation of mutant strains defective in several of these genes. In addition, sequencing of suppressor mutants isolated from anaerobically grown *P. aeruginosa* strains unable to carry out the stringent response might contribute to further understanding of the control of anaerobic colony biofilm growth via ppGpp and DksA. Transcriptome data revealed an involvement of the stringent response in regulation of various infection-relevant cellular processes. Potential roles of ppGpp and DksA in regulation of *P. aeruginosa* motility (type IV pili, swarming), virulence (alkaline proteases, type VI secretion) and metabolism (fatty acid and carbon compound catabolism) also require further investigation.

In this study 129 putative, not previously annotated *P. putida* KT2440 ORF's were identified by *in silico* analysis. As probes for these putative genes were included on Agilent gene expression oligo microarrays used in this study, several of them (e. g. PP4613.1, PP3233.1, PP0201.1) were already shown to be transcribed into mRNA. However, a more global approach is required in order to experimentally verify all putative ORF's, for instance by deep sequencing of mRNA transcripts isolated during diverse growth conditions.

As *P. aeruginosa* nitrate as well as nitrite and nitric oxide reductases were shown to promote *P. putida* KT2440 anaerobic survival, it remains to be determined if this is also true for PAO1 nitrous oxide reductase. In addition, several candidate genes identified *in silico* as present in facultative anaerobic but not obligate aerobic Pseudomonads should be tested regarding their ability to promote active anaerobic growth of KT2440 expressing nitrate reductase or nitrite and nitric oxide reductases. Finally, the role of copper as a potential inhibitor to PAO1 nitrate as well as nitrite and nitric oxide reductases during heterologous expression in *P. putida* requires further investigation.

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Appendix A

***Pseudomonas aeruginosa* PAO1 genes differentially regulated by ppGpp after 15 minutes of SHX treatment of anaerobically grown mid-exponential phase planktonic cultures.** Experimental procedures and data analysis are described elsewhere (3.1.4.1). PA number, gene name, description of encoded proteins and functional classification are according to the "*Pseudomonas* Genome Database". Genes are sorted by their fold induction in wild type treated with SHX ("wt + SHX") compared to wild type ("wt"), fold changes of Δ*relA*Δ*spoT* mutant strain treated with SHX ("Δr/Δs + SHX") compared to wild type treated with SHX ("wt + SHX") are also indicated. Genes marked with an asterisk (*) were differentially regulated in Δ*relA*Δ*spoT* mutant strain treated with SHX compared to Δ*relA*Δ*spoT* mutant strain, but regulation differed more than 2-fold from the fold change expression observed in wild type treated with SHX compared to wild type. Genes marked with a rhombus (#) were regulated only by ppGpp, but not by DksA in a similar experiment (Appendix B).

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
Class I – Induced by ppGpp and upregulated in response to SHX treatment					
7.28	0.28	PA4114 * #	*bltD*	Spermidine acetyltransferase	Putative enzymes
4.99	0.28	PA5460		Putative uncharacterized protein	Hypothetical, unclassified, unknown
4.79	0.24	PA4674 #	*vapI*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
4.63	0.37	PA2788 #		Probable chemotaxis transducer	Chemotaxis
4.59	0.43	PA4130		Probable sulfite or nitrite reductase	Central intermediary metabolism
4.50	0.34	PA3691		Putative uncharacterized protein	Hypothetical, unclassified, unknown
4.08	0.18	PA2622 #	*cspD*	Cold-shock protein CspD	Adaptation & Protection
4.08	0.42	PA5212 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
4.03	0.16	PA3161	*himD*	Integration host factor subunit beta	Transcriptional regulators
3.76	0.29	PA0092		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.76	0.38	PA1657		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.66	0.33	PA0095	*vgr*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.66	0.34	PA4880		Probable bacterioferritin	Central intermediary metabolism
3.61	0.42	PA1517 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.58	0.43	PA5432		Probable acetyltransferase	Putative enzymes
3.56	0.38	PA0093		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.18	0.33	PA5308	*pep4*	Leucine-responsive regulatory protein	Transcriptional regulators

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
3.10	0.23	PA3731		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.05	0.37	PA5191 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.99	0.34	PA0588		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.97	0.37	PA2916		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.89	0.39	PA2883		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.89	0.26	PA4526	*pilB*	Type 4 fimbrial assembly protein PilB	Motility & Attachment
2.85	0.49	PA0271		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.83	0.43	PA1789		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.83	0.34	PA5435	*oadA*	Probable transcarboxylase subunit	Central intermediary metabolism
2.81	0.09	PA3049	*rmf*	Ribosome modulation factor	Translation, post-translational modification, degradation
2.81	0.30	PA4015	*phaJ4*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.79	0.48	PA0269		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.79	0.45	PA2247 #	*bkdA1*	2-oxoisovalerate dehydrogenase subunit alpha	Amino acid biosynthesis & metabolism
2.71	0.30	PA0505		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.69	0.40	PA4919 #	*pncB1*	Nicotinate phosphoribosyltransferase 1	Biosynthesis of cofactors, prosthetic groups & carriers
2.66	0.38	PA0865 #	*hpd*	4-hydroxyphenylpyruvate dioxygenase	Amino acid biosynthesis & metabolism
2.62	0.39	PA1323		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.62	0.49	PA4876 #	*osmE*	Osmotically inducible lipoprotein OsmE	Adaptation & Protection
2.60	0.34	PA1245	*aprX*	AprX protein	Protein secretion & export apparatus
2.60	0.40	PA4296 #	*pprB*	Probable two-component response regulator	Two-component regulatory systems
2.58	0.43	PA0250		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.57	0.48	PA4763 #	*recN*	DNA repair protein RecN	DNA replication, recombination, modification & repair
2.55	0.49	PA0232	*pcaC*	Gamma-carboxymuconolactone decarboxylase	Carbon compound catabolism
2.55	0.44	PA0270		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.45	PA1283		Probable transcriptional regulator	Transcriptional regulators
2.55	0.20	PA3040		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.41	PA3458		Probable transcriptional regulator	Transcriptional regulators
2.53	0.28	PA0094		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.53	0.39	PA1942		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
2.51	0.26	PA3195	*gap*	Glyceraldehyde-3-phosphate dehydrogenase	Energy metabolism
2.50	0.32	PA3919		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.46	0.45	PA0122		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.46	0.33	PA3031		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.46	0.33	PA4793 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.46	0.33	PA5436		Probable biotin carboxylase subunit of transcarboxylase	Central intermediary metabolism
2.45	0.31	PA3257	*prc*	Periplasmic tail-specific protease	Translation, post-translational modification, degradation
2.41	0.39	PA0038		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.39	0.47	PA0656		Probable HIT family protein	Hypothetical, unclassified, unknown
2.38	0.31	PA3792	*leuA*	2-isopropylmalate synthase	Amino acid biosynthesis & metabolism
2.36	0.27	PA3732		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.31	0.43	PA3687	*ppc*	Phosphoenolpyruvate carboxylase	Energy metabolism
2.30	0.44	PA2827	*msrB*	Peptide methionine sulfoxide reductase MsrB	Translation, post-translational modification, degradation
2.27	0.47	PA0469		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.27	0.47	PA0673	*pigB*	Hypothetical PigB	Hypothetical, unclassified, unknown
2.27	0.41	PA1749 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.25	0.44	PA0360 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.25	0.49	PA0563 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.25	0.40	PA2897 #		Probable transcriptional regulator	Transcriptional regulators
2.25	0.34	PA3887	*nhaP*	NhaP	Transport of small molecules
2.23	0.46	PA0096		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.23	0.32	PA5255	*algQ*	Transcriptional regulatory protein AlgQ	Transcriptional regulators
2.22	0.40	PA4614 #	*mscL*	Large-conductance mechanosensitive channel	Transport of small molecules
2.2	0.33	PA0862		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.19	0.41	PA1135	*hchA*	Chaperone protein HchA	Chaperones & heat shock proteins
2.19	0.25	PA4607		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.36	PA0084		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.32	PA0820		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.50	PA4608		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
2.16	0.47	PA1122	*fms*	Probable peptide deformylase	Translation, post-translational modification, degradation
2.16	0.48	PA5329 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.13	0.46	PA0082 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.13	0.40	PA4026		Probable acetyltransferase	Putative enzymes
2.13	0.40	PA4657 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.11	0.40	PA0200		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.11	0.39	PA0713 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.11	0.48	PA4530 #		UPF0243 zinc-binding protein PA4530	Hypothetical, unclassified, unknown
2.07	0.41	PA3349 #		Probable chemotaxis protein	Chemotaxis
2.07	0.49	PA5274 #	*rnk*	nucleoside diphosphate kinase regulator	Transcriptional regulators
2.04	0.49	PA2737		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.33	PA3662		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.37	PA3684 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.35	PA3730 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.03	0.33	PA3183	*zwf*	Glucose-6-phosphate 1-dehydrogenase	Energy metabolism
2.01	0.48	PA1818	*speC*	Probable Orn/Arg/Lys decarboxylase	Amino acid biosynthesis & metabolism
Class II – repressed by ppGpp and downregulated in response to SHX					
0.49	2.35	PA0430	*metF*	Methylenetetrahydrofolate reductase	Amino acid biosynthesis & metabolism
0.49	2.03	PA0576	*rpoD*	RNA polymerase sigma factor RpoD	Transcriptional regulators
0.49	2.73	PA2957		Probable transcriptional regulator	Transcriptional regulators
0.49	2.36	PA4263 #	*rplC*	50S ribosomal protein L3	Translation, post-translational modification, degradation
0.49	3.39	PA5118	*thiI*	Thiamine biosynthesis protein ThiI	Biosynthesis of cofactors, prosthetic groups & carriers
0.48	2.45	PA0541		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.07	PA0768 #	*lepB*	Signal peptidase I	Protein secretion/export apparatus
0.48	2.17	PA2817		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.87	PA4935	*rpsF*	30S ribosomal protein S6	Translation, post-translational modification, degradation
0.48	2.03	PA5139		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.51	PA5300	*cycB*	Cytochrome c5	Energy metabolism
0.48	2.39	PA5502		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.47	2.04	PA4665	*prfA*	Peptide chain release factor 1	Translation, post-translational modification, degradation
0.46	3.29	PA2042	*sstT*	Serine/threonine transporter SstT	Transport of small molecules
0.46	2.16	PA2876	*pyrF*	Orotidine 5'-phosphate decarboxylase	Nucleotide biosynthesis & metabolism
0.46	2.71	PA3111	*folC*	Folylpolyglutamate synthetase	Biosynthesis of cofactors, prosthetic groups & carriers
0.46	2.89	PA4053	*ribH*	6,7-dimethyl-8-ribityllumazine synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.46	2.17	PA4261 #	*rplW*	50S ribosomal protein L23	Translation, post-translational modification, degradation
0.46	2.01	PA4741	*rpsO*	30S ribosomal protein S15	Translation, post-translational modification, degradation
0.46	3.89		tRNA-Tyr		Translation, post-translational modification, degradation
0.45	2.79	PA0916 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.45	2.91	PA1757	*thrH*	Homoserine kinase	Amino acid biosynthesis & metabolism
0.45	2.20	PA2637	*nuoA*	NADH-quinone oxidoreductase subunit A	Energy metabolism
0.45	2.27	PA3816	*cysE*	O-acetylserine synthase	Amino acid biosynthesis & metabolism
0.45	2.73	PA3817		Probable methyltransferase	Putative enzymes
0.45	2.16	PA5005		Probable carbamoyl transferase	Putative enzymes
0.45	2.46	PA5316	*rpmB*	50S ribosomal protein L28	Translation, post-translational modification, degradation
0.45	2.36	PA5504		Probable permease of ABC transporter	Transport of small molecules
0.44	3.48	PA1228		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	2.11	PA2999	*nqrA*	Na(+)-translocating NADH-quinone reductase subunit A	Energy metabolism
0.44	2.01	PA3391 #	*nosR*	Regulatory protein NosR	Transcriptional regulators
0.44	2.43	PA4007	*proA*	Gamma-glutamyl phosphate reductase	Amino acid biosynthesis & metabolism
0.44	2.20	PA4291		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	3.20	PA4745	*nusA*	N utilization substance protein A	Transcription, RNA processing & degradation
0.44	2.10	PA5351	*rubA1*	Rubredoxin 1	Carbon compound catabolism
0.43	2.64	PA0381	*thiG*	Thiazole biosynthesis protein ThiG	Biosynthesis of cofactors, prosthetic groups & carriers
0.43	2.91	PA0431		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	3.86	PA1687	*speE1*	Spermidine synthase 1	Amino acid biosynthesis & metabolism
0.43	2.22	PA2800	*vacJ*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	6.63	PA3179	*rluB*	Ribosomal large subunit pseudouridine synthase B	Translation, post-translational modification, degradation
0.43	2.03	PA3263	*rdgC*	Recombination-associated protein RdgC	DNA replication, recombination, modification & repair

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.43	3.16	PA3742	*rplS*	50S ribosomal protein L19	Translation, post-translational modification, degradation
0.43	2.28	PA4262 #	*rplD*	50S ribosomal protein L4	Translation, post-translational modification, degradation
0.43	2.06	PA4431		Probable iron-sulfur protein	Putative enzymes
0.43	2.62	PA4456		Probable ATP-binding component of ABC transporter	Transport of small molecules
0.43	3.14	PA4519	*speC*	Ornithine decarboxylase	Amino acid biosynthesis & metabolism
0.43	2.45	PA4719		Probable transporter	Transport of small molecules
0.43	2.62	PA5130		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	3.27	PA5167		Probable c4-dicarboxylate-binding protein	Transport of small molecules
0.42	2.55	PA0974		Periplasmic protein	Hypothetical, unclassified, unknown
0.42	2.45	PA3746	*ffh*	Signal recognition particle protein Ffh	Protein secretion/export apparatus
0.42	2.58	PA5503	*metN2*	Methionine import ATP-binding protein MetN2	Transport of small molecules
0.41	2.23	PA0382	*trmB*	tRNA (guanine-N(7)-)-methyltransferase	Translation, post-translational modification, degradation
0.41	2.51	PA3168	*gyrA*	DNA gyrase subunit A	DNA replication, recombination, modification & repair
0.41	4.44	PA3770	*guaB*	Inosine-5'-monophosphate dehydrogenase	Nucleotide biosynthesis & metabolism
0.41	3.03	PA4433	*rplM*	50S ribosomal protein L13	Translation, post-translational modification, degradation
0.41	3.03	PA4645	*hpt*	Probable purine/pyrimidine phosphoribosyl transferase	Nucleotide biosynthesis & metabolism
0.41	2.19	PA4753		Probable RNA-binding protein	Hypothetical, unclassified, unknown
0.41	4.23	PA5048		Probable nuclease	Putative enzymes
0.41	2.66	PA5129	*grx*	Glutaredoxin	Energy metabolism
0.41	2.36	PA5426	*purE*	Phosphoribosylaminoimidazole carboxylase catalytic subunit	Nucleotide biosynthesis & metabolism
0.40	2.23	PA1796	*folD*	Bifunctional protein FolD	Biosynthesis of cofactors, prosthetic groups & carriers
0.40	2.69	PA1800	*tig*	Trigger factor	Cell division
0.40	2.27	PA1815	*rnhA*	Ribonuclease HI	DNA replication, recombination, modification & repair
0.40	2.31	PA2626	*trmU*	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	Translation, post-translational modification, degradation
0.40	2.48	PA3001		Probable glyceraldehyde-3-phosphate dehydrogenase	Putative enzymes
0.40	4.23	PA3655	*tsf*	Elongation factor Ts	Translation, post-translational modification, degradation
0.40	2.06	PA4237	*rplQ*	50S ribosomal protein L17	Translation, post-translational modification, degradation
0.40	2.57	PA4632		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	2.08	PA4666	*hemA*	Glutamyl-tRNA reductase	Translation, post-translational modification, degradation

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.40	2.55	PA4724	*gluQ*	Glutamyl-Q tRNA synthetase	Translation, post-translational modification, degradation
0.40	3.78	PA4742	*truB*	tRNA pseudouridine synthase B	Translation, post-translational modification, degradation
0.39	2.06	PA3808		Uncharacterized protein	Hypothetical, unclassified, unknown
0.39	3.16	PA4005		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.39	2.69	PA4812	*fdnG*	Formate dehydrogenase-O, major subunit	Energy metabolism
0.38	4.06	PA0945	*purM*	Phosphoribosylformylglycinamidine cyclo-ligase	Nucleotide biosynthesis & metabolism
0.38	3.18	PA4006	*nadD*	Probable nicotinate-nucleotide adenylyltransferase	Biosynthesis of cofactors, prosthetic groups & carriers
0.38	2.11	PA4810 #	*fdnI*	Nitrate-inducible formate dehydrogenase, gamma subunit	Energy metabolism
0.38	2.50	PA5201		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.37	2.03	PA1012		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.37	2.19	PA2023	*galU*	UTP--glucose-1-phosphate uridylyltransferase	Central intermediary metabolism
0.37	3.41	PA3980	*miaB*	UPF0004 protein PA3980	Translation, post-translational modification, degradation
0.37	3.03	PA4004		UPF0247 protein PA4004	Hypothetical, unclassified, unknown
0.37	2.97	PA4640	*mqo2*	Probable malate:quinone oxidoreductase 2	Energy metabolism
0.37	2.45	PA4723 #	*dksA*	Multicopy mutation suppressor DksA	Transcriptional regulators
0.37	3.56	PA5440		Probable peptidase	Translation, post-translational modification, degradation
0.36	3.20	PA0380		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.36	3.25	PA0767	*lepA*	GTP-binding protein LepA	Protein secretion/export apparatus
0.36	2.17	PA3169	*mtnA*	Probable methylthioribose-1-phosphate isomerase	Translation, post-translational modification, degradation
0.36	3.14	PA4268	*rpsL*	30S ribosomal protein S12	Translation, post-translational modification, degradation
0.36	2.48	PA4684		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.36	3.41	PA4853	*fis*	Putative fis-like DNA-binding protein	Transcriptional regulators
0.35	2.73	PA1475	*ccmA*	Cytochrome c biogenesis ATP-binding export protein ccmA	Transport of small molecules
0.35	16.34	PA2840	*deaD*	Probable ATP-dependent RNA helicase	Transcription, RNA processing & degradation
0.35	3.14	PA2901		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.35	2.93	PA4333	*fumA*	Probable fumarase	Energy metabolism
0.35	5.03	PA4746		UPF0090 protein PA4746	Hypothetical, unclassified, unknown
0.34	4.82	PA0654	*speD*	S-adenosylmethionine decarboxylase proenzyme	Central intermediary metabolism
0.34	2.81	PA0968		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.34	3.12	PA2740	*pheS*	Phenylalanyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation
0.34	5.17	PA2971		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	2.48	PA2983		Probable tolQ-type transport protein	Transport of small molecules
0.34	3.61	PA3653	*frr*	Ribosome recycling factor	Translation, post-translational modification, degradation
0.34	2.93	PA4636		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	3.92	PA4744	*infB*	Translation initiation factor IF-2	Translation, post-translational modification, degradation
0.34	2.53	PA4854	*purH*	Bifunctional purine biosynthesis protein PurH	Nucleotide biosynthesis & metabolism
0.34	3.05	PA5340		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.33	2.81	PA2453		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.33	2.08	PA3645	*fabZ*	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase	Fatty acid & phospholipid metabolism
0.33	4.92	PA3654	*pyrH*	Uridylate kinase	Nucleotide biosynthesis & metabolism
0.33	2.62	PA4747	*secG*	Protein-export membrane protein SecG	Protein secretion/export apparatus
0.33	2.17	PA5053	*hslV*	ATP-dependent protease HslV	Chaperones & heat shock proteins
0.32	2.43	PA1192		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.32	3.05	PA3308	*rapA*	RNA polymerase-associated protein RapA	Transcription, RNA processing & degradation
0.32	2.64	PA3821	*secD*	Protein-export membrane protein SecD	Protein secretion/export apparatus
0.32	4.41	PA5049	*rpmE*	50S ribosomal protein L31	Translation, post-translational modification, degradation
0.31	2.68	PA0888	*aotJ*	Arginine and ornithine binding protein	Transport of small molecules
0.31	2.23	PA3636	*kdsA*	2-dehydro-3-deoxyphosphooctonate aldolase	Cell wall, LPS & capsule
0.31	3.84	PA3807	*ndk*	Nucleoside diphosphate kinase	Nucleotide biosynthesis & metabolism
0.31	3.78	PA3824	*queA*	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	Translation, post-translational modification, degradation
0.31	3.05	PA4052	*nusB*	N utilization substance protein B homolog	Transcription, RNA processing & degradation
0.31	3.76	PA5479	*gltP*	Proton-glutamate symporter	Transport of small molecules
0.30	2.35	PA1478	*ccmD*	Heme exporter protein D	Transport of small molecules
0.30	2.04	PA2739	*pheT*	Phenylalanyl-tRNA synthetase beta chain	Translation, post-translational modification, degradation
0.30	3.48	PA3701	*prfB*	Peptide chain release factor 2	Translation, post-translational modification, degradation
0.30	3.86	PA3823	*tgt*	Queuine tRNA-ribosyltransferase	Translation, post-translational modification, degradation
0.30	3.68	PA4480	*mreC*	Rod shape-determining protein MreC	Cell division
0.30	3.97	PA4933		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0,30	2.50		tRNA_Leu		Translation, post-translational modification, degradation
0.29	2.57	PA0775		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.29	4.03	PA1790		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.29	2.77	PA1812	*mltD*	Membrane-bound lytic murein transglycosylase D	Cell wall, LPS & capsule
0.29	2.36	PA4243 #	*secY*	Preprotein translocase subunit SecY	Protein secretion/export apparatus
0.29	3.97	PA4438		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.29	4.14	PA4852	*dusB*	tRNA-dihydrouridine synthase B	Translation, post-translational modification, degradation
0.28	3.78	PA1183	*dctA2*	C4-dicarboxylate transport protein 2	Transport of small molecules
0.28	4.32	PA3769	*guaA*	GMP synthase	Nucleotide biosynthesis & metabolism
0.28	3.03	PA3811	*hscB*	Co-chaperone protein HscB homolog	Chaperones & heat shock proteins
0.28	2.20	PA4685		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.28	2.57		tRNA_Asn		Translation, post-translational modification, degradation
0.28	3.18		tRNA_His		Translation, post-translational modification, degradation
0.28	3,78		tRNA_Val		Translation, post-translational modification, degradation
0.27	2.53	PA3245	*minE*	Cell division topological specificity factor	Cell division
0.27	2.39	PA3313		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.27	3.68	PA3652	*uppS*	Undecaprenyl pyrophosphate synthetase	Cell wall, LPS & capsule
0.27	2.91	PA4768 #	*smpB*	SsrA-binding protein	Translation, post-translational modification, degradation
0.27	2.73	PA4770	*lldP*	L-lactate permease	Transport of small molecules
0.27	3.25	PA5192	*pckA*	Phosphoenolpyruvate carboxykinase	Energy metabolism
0.26	2.75	PA0760		UPF0350 protein PA0760	Hypothetical, unclassified, unknown
0.26	2.48	PA2798		Probable two-component response regulator	Two-component regulatory systems
0.26	3.97	PA2851	*efp*	Elongation factor P	Translation, post-translational modification, degradation
0.26	2.73	PA3162	*rpsA*	30S ribosomal protein S1	Translation, post-translational modification, degradation
0.25	2.41	PA4031	*ppa*	Inorganic pyrophosphatase	Central intermediary metabolism
0.25	4.66	PA5117	*typA*	Regulatory protein TypA	Adaptation & Protection
0.25	5.43	PA5286		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.24	4.17	PA1554	*ccoN*	Probable cytochrome oxidase subunit	Energy metabolism
0.24	2.85	PA4255	*rpmC*	50S ribosomal protein L29	Translation, post-translational modification, degradation

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.24	4.86	PA4292		Probable phosphate transporter	Transport of small molecules
0.24	2.17	PA4429 #		Probable cytochrome c1	Energy metabolism
0.24	5.74	PA4743	*rbfA*	Ribosome-binding factor A	Translation, post-translational modification, degradation
0.23	4.66	PA3641		Probable amino acid permease	Transport of small molecules
0.23	3.34	PA3700	*lysS*	Lysyl-tRNA synthetase	Translation, post-translational modification, degradation
0.23	6.19	PA3818	*suhB*	Inositol-1-monophosphatase	Translation, post-translational modification, degradation
0.23	4.96	PA4432	*rpsI*	30S ribosomal protein S9	Translation, post-translational modification, degradation
0.23	4.11		tRNA_Ala		Translation, post-translational modification, degradation
0.22	2.11	PA0667		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.22	5.46	PA2970	*rpmF*	50S ribosomal protein L32	Translation, post-translational modification, degradation
0.22	3.71	PA3806		UPF0063 protein PA3806	Hypothetical, unclassified, unknown
0.22	4.23	PA4563	*rpsT*	30S ribosomal protein S20	Translation, post-translational modification, degradation
0.22	6.28		tRNA_Trp		Translation, post-translational modification, degradation
0.21	5.28	PA4569	*ispB*	Octaprenyl-diphosphate synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.21	3.68	PA4673		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.21	4.66	PA5298	*xpt*	Xanthine phosphoribosyltransferase	Nucleotide biosynthesis & metabolism
0.21	2.39	PA5315	*rpmG*	50S ribosomal protein L33	Translation, post-translational modification, degradation
0.20	6.73	PA4481	*mreB*	Rod shape-determining protein MreB	Cell division
0.20	2.71	PA5445	*psecoA*	Probable coenzyme A transferase	Putative enzymes
0.20	5.66	PA5491		Probable cytochrome	Energy metabolism
0.20	3.23	PA1553 * #	*fixO*	Probable cytochrome c oxidase subunit	Energy metabolism
0.19	3.16	PA2638	*nuoB*	NADH-quinone oxidoreductase subunit B	Energy metabolism
0.19	1.29	PA4307 *	*pctC*	Chemotactic transducer PctC	Chemotaxis
0.18	7.41	PA3744	*rimM*	16S rRNA-processing protein RimM	Transcription, RNA processing & degradation
0.18	5.58	PA4276	*secE*	Preprotein translocase subunit SecE	Protein secretion/export apparatus
0.18	8.11	PA4748	*tpiA*	Triosephosphate isomerase	Energy metabolism
0.18	2.11	PA1837 *		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.17	7.06	PA2629	*purB*	Adenylosuccinate lyase	Nucleotide biosynthesis & metabolism
0.17	3.27	PA4430		Probable cytochrome b	Energy metabolism

FC wt + SHX vs. wt	FC Δr/Δs + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.16	5.54	PA4275	*nusG*	Transcription antitermination protein NusG	Transcription, RNA processing & degradation
0.16	6.45	PA4670	*prs*	Ribose-phosphate pyrophosphokinase	Nucleotide biosynthesis & metabolism
0.16	3.07	PA1838 *	*cysI*	Sulfite reductase	Central intermediary metabolism
0.15	8.17	PA0578		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.15	10.41	PA3743	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	Translation, post-translational modification, degradation
0.15	5.50	PA4672	*pth*	Peptidyl-tRNA hydrolase	Translation, post-translational modification, degradation
0.15	4.38		tRNA_Lys		Translation, post-translational modification, degradation
0.13	7.41	PA0579	*rpsU*	30S ribosomal protein S21	Translation, post-translational modification, degradation
0.13	10.20	PA3745	*rpsP*	30S ribosomal protein S16	Translation, post-translational modification, degradation
0.13	7.52		tRNA_Gln		Translation, post-translational modification, degradation
0.10	10.63		tRNA_Ile		Translation, post-translational modification, degradation
0.07	10.70	PA2619	*infA*	Translation initiation factor IF-1	Translation, post-translational modification, degradation
0.06	25.63		tRNA_Gly		Translation, post-translational modification, degradation
Class III – induced by ppGpp, downregulated in response to SHX treatment					
0.43	0.38	PA3155 * #	*wbpE*	Probable aminotransferase WbpE	Cell wall, LPS & capsule

Appendix B

***Pseudomonas aeruginosa* PAO1 genes differentially regulated by DksA after 15 minutes of SHX treatment of anaerobically grown mid-exponential phase planktonic cultures.** Experimental procedures and data analysis are described elsewhere (3.1.4.1). PA number, gene name, description of encoded proteins and functional classification are according to the "*Pseudomonas* Genome Database". Genes are sorted by their fold induction in wild type treated with SHX ("wt + SHX") compared to wild type ("wt"), fold changes of Δ*dksA* mutant strain treated with SHX ("Δd + SHX") compared to wild type treated with SHX ("wt + SHX") are also indicated. Genes marked with a rhombus (#) were regulated only by DksA, but not by ppGpp in a similar experiment (Appendix A).

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
Class I – Induced by DksA in response to SHX treatment					
9.45	0.08	PA0263 #	*hcpA*	Major exported protein	Secreted factors (toxins, enzymes, alginate)
7.57	0.13	PA4133 #		Cytochrome c oxidase subunit	Energy metabolism
6.77	0.26	PA4131 #		Probable iron-sulfur protein	Putative enzymes
4.99	0.20	PA5460		Putative uncharacterized protein	Hypothetical, unclassified, unknown
4.59	0.31	PA4130		Probable sulfite or nitrite reductase	Central intermediary metabolism
4.50	0.48	PA3691		Putative uncharacterized protein	Hypothetical, unclassified, unknown
4.03	0.18	PA3161	*himD*	Integration host factor subunit beta	Transcriptional regulators
3.76	0.45	PA0092		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.76	0.20	PA1657		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.66	0.24	PA0095	*vgr*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.66	0.34	PA4880		Probable bacterioferritin	Central intermediary metabolism
3.58	0.40	PA0224 #		Probable aldolase	Putative enzymes
3.58	0.13	PA2381 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.58	0.31	PA5432		Probable acetyltransferase	Putative enzymes
3.56	0.43	PA0093		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.18	0.45	PA5308	*pep4*	Leucine-responsive regulatory protein	Transcriptional regulators
3.10	0.25	PA3731		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.03	0.36	PA2031 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.03	0.35	PA4881 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
2.99	0.21	PA0588		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.97	0.29	PA2916		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.89	0.46	PA2883		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.89	0.40	PA4526	*pilB*	Type 4 fimbrial assembly protein PilB	Motility & Attachment
2.85	0.29	PA0271		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.85	0.25	PA1247 #	*aprE*	Alkaline protease secretion protein AprE	Secreted factors (toxins, enzymes, alginate)
2.83	0.36	PA1789		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.83	0.21	PA5435	*oadA*	Probable transcarboxylase subunit	Central intermediary metabolism
2.81	0.13	PA3049	*rmf*	Ribosome modulation factor	Translation, post-translational modification, degradation
2.81	0.31	PA4015	*phaJ4*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.81	0.28	PA4898 #	*opdK*	Probable porin	Transport of small molecules
2.79	0.29	PA0135 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.79	0.34	PA0269		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.77	0.42	PA2190 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.71	0.46	PA0505		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.62	0.49	PA1323		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.60	0.24	PA1245	*aprX*	AprX protein	Protein secretion & export apparatus
2.58	0.36	PA0250		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.23	PA0232	*pcaC*	Gamma-carboxymuconolactone decarboxylase	Carbon compound catabolism
2.55	0.28	PA0270		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.27	PA1283		Probable transcriptional regulator	Transcriptional regulators
2.55	0.16	PA3040		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.43	PA3458		Probable transcriptional regulator	Transcriptional regulators
2.53	0.37	PA0094		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.53	0.34	PA1942		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.53	0.40	PA4211 #	*phzB1*	Phenazine biosynthesis protein PhzB1	Secreted factors (toxins, enzymes, alginate)
2.51	0.36	PA0206 #	*potA1*	Spermidine/putrescine import ATP-binding protein potA 1	Transport of small molecules
2.51	0.22	PA3195	*gap*	Glyceraldehyde-3-phosphate dehydrogenase	Energy metabolism
2.50	0.25	PA3919		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
2.46	0.38	PA0122		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.46	0.35	PA0289 #	*gpuR*	Transcriptional activator GpuR	Transcriptional regulators
2.46	0.43	PA3031		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.46	0.21	PA5436		Probable biotin carboxylase subunit of transcarboxylase	Central intermediary metabolism
2.45	0.30	PA3257	*prc*	Periplasmic tail-specific protease	Translation, post-translational modification, degradation
2.43	0.42	PA0160 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.41	0.43	PA0038		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.41	0.30	PA3584 #	*glpD*	Glycerol-3-phosphate dehydrogenase	Energy metabolism
2.39	0.28	PA0656		Probable HIT family protein	Hypothetical, unclassified, unknown
2.38	0.37	PA3792	*leuA*	2-isopropylmalate synthase	Amino acid biosynthesis & metabolism
2.36	0.38	PA0261 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.36	0.44	PA2146 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.36	0.31	PA3732		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.35	0.31	PA4915 #		Probable chemotaxis transducer	Chemotaxis
2.31	0.45	PA0447 #	*gcdH*	Glutaryl-CoA dehydrogenase	Fatty acid & phospholipid metabolism
2.31	0.36	PA3687	*ppc*	Phosphoenolpyruvate carboxylase	Energy metabolism
2.30	0.32	PA1362 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.30	0.48	PA1511 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.30	0.48	PA2827	*msrB*	Peptide methionine sulfoxide reductase MsrB	Translation, post-translational modification, degradation
2.30	0.24	PA5415 #	*glyA1*	Serine hydroxymethyltransferase 1	Amino acid biosynthesis & metabolism
2.30	0.48	PA5566 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.28	0.49	PA2248 #	*bkdA2*	2-oxoisovalerate dehydrogenase subunit beta	Amino acid biosynthesis & metabolism
2.28	0.41	PA4129 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.27	0.45	PA0469		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.27	0.47	PA0673	*pigB*	Hypothetical PigB	Hypothetical, unclassified, unknown
2.25	0.27	PA3887	*nhaP*	Na/H Antiporter NhaP	Transport of small molecules
2.23	0.34	PA0096		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.23	0.38	PA0234 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.23	0.39	PA5255	*algQ*	Transcriptional regulatory protein AlgQ	Transcriptional regulators

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
2.20	0.31	PA0862		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.19	0.37	PA1135	*hchA*	Chaperone protein HchA	Chaperones & heat shock proteins
2.19	0.37	PA3566 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.19	0.27	PA4607		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.48	PA0084		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.44	PA0181 #		Probable transcriptional regulator	Transcriptional regulators
2.17	0.41	PA0820		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.46	PA3729 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.44	PA4107 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.45	PA4608		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.43	PA5356 #	*glcC*	Transcriptional regulator GlcC	Transcriptional regulators
2.17	0.43	PA5395 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.16	0.47	PA1122	*fms*	Probable peptide deformylase	Translation, post-translational modification, degradation
2.13	0.38	PA0205 #		Probable permease of ABC transporter	Transport of small molecules
2.13	0.36	PA2016 #	*liuR*	Regulatory gene of liuABCDE cluster	Transcriptional regulators
2.13	0.41	PA4026		Probable acetyltransferase	Putative enzymes
2.11	0.30	PA0200		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.10	0.40	PA0107 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.10	0.45	PA3520 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.07	0.48	PA0176 #	*aer2*	Aerotaxis transducer Aer2	Chemotaxis
2.07	0.35	PA1246 #	*aprD*	Alkaline protease secretion ATP-binding protein	Secreted factors (toxins, enzymes, alginate)
2.06	0.45	PA1656 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.46	PA0192 #		Probable TonB-dependent receptor	Transport of small molecules
2.04	0.37	PA0193 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.42	PA2737		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.34	PA3662		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.30	PA3730 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.48	PA5352 #	*glcG*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.04	0.44	PA5538 #	*amiA*	N-acetylmuramoyl-L-alanine amidase	Cell wall, LPS & capsule

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
2.03	0.44	PA0151 #		Probable TonB-dependent receptor	Transport of small molecules
2.03	0.40	PA0325 #		Probable permease of ABC transporter	Transport of small molecules
2.03	0.44	PA0978 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.03	0.40	PA3183	*zwf*	Glucose-6-phosphate 1-dehydrogenase	Energy metabolism
2.01	0.49	PA1248 #	*aprF*	Alkaline protease secretion protein AprF	Secreted factors (toxins, enzymes, alginate)
2.01	0.43	PA1818	*speC*	Probable Orn/Arg/Lys decarboxylase	Amino acid biosynthesis & metabolism
2.01	0.47	PA2149 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
Class II – Repressed by DksA in response to SHX treatment					
0.49	2.28	PA0576	*rpoD*	RNA polymerase sigma factor RpoD	Transcriptional regulators
0.49	2.23	PA1574 #		UPF0345 protein PA1574	Hypothetical, unclassified, unknown
0.49	3.61	PA2957		Probable transcriptional regulator	Transcriptional regulators
0.49	2.22	PA3644 #	*lpxA*	UDP-N-acetylglucosamine acyltransferase	Cell wall, LPS & capsule
0.49	3.22	PA0430	*metF*	Methylenetetrahydrofolate reductase	Amino acid biosynthesis & metabolism
0.49	2.16	PA4051 #	*thiL*	Thiamine monophosphate kinase	Biosynthesis of cofactors, prosthetic groups & carriers
0.49	4.94	PA5118	*thiI*	Thiamine biosynthesis protein ThiI	Biosynthesis of cofactors, prosthetic groups & carriers
0.48	2.18	PA0925 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.36	PA3011 #	*topA*	DNA topoisomerase 1	DNA replication, recombination, modification & repair
0.48	2.21	PA4454 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.10	PA4728 #	*folK*	2-amino-4-hydroxy-6-hydroxymethyldihydropteridinepyrophosphokinase	Biosynthesis of cofactors, prosthetic groups & carriers
0.48	3.61	PA4935	*rpsF*	30S ribosomal protein S6	Translation, post-translational modification, degradation
0.48	3.71	PA5300	*cycB*	Cytochrome c5	Energy metabolism
0.48	2.80	PA5490 #	*cc4*	Cytochrome c4 precursor	Energy metabolism
0.48	4.11	PA2817		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.41	PA2975 #	*rluC*	Ribosomal large subunit pseudouridine synthase C	Translation, post-translational modification, degradation
0.48	3.44	PA5502		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	3.10	PA0541		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	3.43	PA1045 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.63	PA5139		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.47	3.02	PA5425 #	*purK*	Phosphoribosylaminoimidazole carboxylase ATPase subunit	Nucleotide biosynthesis & metabolism
0.47	2.56	PA0926 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.47	2.13	PA3632 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.47	2.03	PA3620 #	*mutS*	DNA mismatch repair protein MutS	DNA replication, recombination, modification & repair
0.47	2.53	PA4665	*prfA*	Peptide chain release factor 1	Translation, post-translational modification, degradation
0.46	2.33	PA1768 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.46	2.66	PA2042	*sstT*	Serine/threonine transporter SstT	Transport of small molecules
0.46	2.42	PA4241 #	*rpsM*	30S ribosomal protein S13	Translation, post-translational modification, degradation
0.46	2.33	PA4855 #	*purD*	Phosphoribosylamine--glycine ligase	Biosynthesis of cofactors, prosthetic groups & carriers
0.46	2.77	PA4741	*rpsO*	30S ribosomal protein S15	Translation, post-translational modification, degradation
0.46	3.56	PA5565 #	*gidA*	glucose-inhibited division protein A	Cell division
0.46	2.57	PA2876	*pyrF*	Orotidine 5'-phosphate decarboxylase	Nucleotide biosynthesis & metabolism
0.46	2.21	PA3111	*folC*	Folylpolyglutamate synthetase	Biosynthesis of cofactors, prosthetic groups & carriers
0.46	2.34	PA3996 #	*lipA*	Lipoyl synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.46	3.94	PA4053	*ribH*	6,7-dimethyl-8-ribityllumazine synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.46	6.85		tRNA_Tyr		Translation, post-translational modification, degradation
0.45	4.35	PA1757	*thrH*	Homoserine kinase	Amino acid biosynthesis & metabolism
0.45	2.28	PA4452 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.45	2.67	PA5005		Probable carbamoyl transferase	Putative enzymes
0.45	3.42	PA5316	*rpmB*	50S ribosomal protein L28	Translation, post-translational modification, degradation
0.45	2.09	PA2637	*nuoA*	NADH-quinone oxidoreductase subunit A	Energy metabolism
0.45	3.39	PA3816	*cysE*	O-acetylserine synthase	Amino acid biosynthesis & metabolism
0.45	5.00	PA3817		Probable methyltransferase	Translation, post-translational modification, degradation
0.45	2.28	PA5504		Probable permease of ABC transporter	Transport of small molecules
0.45	2.49		tRNA_Phe		Translation, post-translational modification, degradation
0.44	3.79	PA4007	*proA*	Gamma-glutamyl phosphate reductase	Biosynthesis of cofactors, prosthetic groups & carriers
0.44	2.10	PA2998 #	*nqrB*	Na(+)-translocating NADH-quinone reductase subunit B	Energy metabolism
0.44	2.05	PA3246 #	*rluA*	Pseudouridine synthase	Translation, post-translational modification, degradation
0.44	3.47	PA5351	*rubA1*	Rubredoxin 1	Carbon compound catabolism

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.44	13.24	PA1228		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	2.74	PA2999	*nqrA*	Na(+)-translocating NADH-quinone reductase subunit A	Energy metabolism
0.44	2.73	PA4291		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	3.91	PA4745	*nusA*	N utilization substance protein A	Transcription, RNA processing & degradation
0.43	3.23	PA0381	*thiG*	Thiazole biosynthesis protein ThiG	Biosynthesis of cofactors, prosthetic groups & carriers
0.43	2.59	PA3263	*rdgC*	Recombination-associated protein RdgC	DNA replication, recombination, modification & repair
0.43	3.69	PA4456	*yrbF*	Probable ATP-binding component of ABC transporter	Transport of small molecules
0.43	3.09	PA4676 #	*yadF*	Carbonic anhydrase	Putative enzymes
0.43	2.65	PA5167		Probable c4-dicarboxylate-binding protein	Transport of small molecules
0.43	2.46	PA5557 #	*atpH*	ATP synthase delta chain	Energy metabolism
0.43	7.83	PA1687	*speE1*	Spermidine synthase 1	Amino acid biosynthesis & metabolism
0.43	2.32	PA3018 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	2.04	PA4431		Probable iron-sulfur protein	Putative enzymes
0.43	5.87	PA4719		Probable transporter	Transport of small molecules
0.43	4.08	PA0431		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	2.92	PA2800	*vacJ*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	17.93	PA3179	*rluB*	Ribosomal large subunit pseudouridine synthase B	Translation, post-translational modification, degradation
0.43	4.53	PA3742	*rplS*	50S ribosomal protein L19	Translation, post-translational modification, degradation
0.43	7.04	PA4519	*speC*	Ornithine decarboxylase	Amino acid biosynthesis & metabolism
0.43	3.69	PA5130		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.42	5.18	PA0045 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.42	2.89	PA3746	*ffh*	Signal recognition particle protein Ffh	Protein secretion & export apparatus
0.42	2.30	PA3975 #	*thiD*	Phosphomethylpyrimidine kinase	Biosynthesis of cofactors, prosthetic groups & carriers
0.42	3.47	PA0974		Periplasmic protein	Hypothetical, unclassified, unknown
0.42	3.13	PA5503	*metN2*	Methionine import ATP-binding protein MetN2	Transport of small molecules
0.42	2.76	PA4727 #	*pcnB*	Poly(A) polymerase	Transcription, RNA processing & degradation
0.41	5.15	PA4645	*hpt*	Probable purine/pyrimidine phosphoribosyl transferase	Nucleotide biosynthesis & metabolism
0.41	8.09	PA5048		Probable nuclease	Putative enzymes
0.41	3.34	PA5129	*grx*	Glutaredoxin	Energy metabolism

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.41	3.84	PA5426	*purE*	Phosphoribosylaminoimidazole carboxylase catalytic subunit	Nucleotide biosynthesis & metabolism
0.41	2.52	PA5558 #	*atpF*	ATP synthase B chain	Energy metabolism
0.41	2.17	PA0382	*trmB*	tRNA (guanine-N(7)-)-methyltransferase	Translation, post-translational modification, degradation
0.41	3.38	PA4753		Probable RNA-binding protein	Hypothetical, unclassified, unknown
0.41	2.28	PA5128 #	*secB*	Protein-export protein SecB	Protein secretion & export apparatus
0.41	2.61	PA3168	*gyrA*	DNA gyrase subunit A	DNA replication, recombination, modification & repair
0.41	5.81	PA3770	*guaB*	Inosine-5'-monophosphate dehydrogenase	Nucleotide biosynthesis & metabolism
0.41	3.73	PA4433	*rplM*	50S ribosomal protein L13	Translation, post-translational modification, degradation
0.41	2.28	PA2321 #	*gnuK*	Gluconate kinase	Carbon compound catabolism
0.40	3.01	PA1800	*tig*	Trigger factor	Translation, post-translational modification, degradation
0.40	6.54	PA3655	*tsf*	Elongation factor Ts	Translation, post-translational modification, degradation
0.40	2.40	PA4237	*rplQ*	50S ribosomal protein L17	Translation, post-translational modification, degradation
0.40	4.96	PA4742	*truB*	tRNA pseudouridine synthase B	Translation, post-translational modification, degradation
0.40	4.64	PA4724	*gluQ*	Glutamyl-Q tRNA(Asp) synthetase	Translation, post-translational modification, degradation
0.40	2.35	PA3001		Probable glyceraldehyde-3-phosphate dehydrogenase	Putative enzymes
0.40	2.33	PA4449 #	*hisG*	ATP phosphoribosyltransferase	Amino acid biosynthesis & metabolism
0.40	3.1	PA4632		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	3.31	PA1796	*folD*	Bifunctional protein FolD	Biosynthesis of cofactors, prosthetic groups & carriers
0.40	3.72	PA1815	*rnhA*	Ribonuclease HI	DNA replication, recombination, modification & repair
0.40	2.39	PA2626	*trmU*	Probable tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	Translation, post-translational modification, degradation
0.40	3.71	PA4666	*hemA*	Glutamyl-tRNA reductase	Translation, post-translational modification, degradation
0.39	2.19	PA2973 #		Probable peptidase	Translation, post-translational modification, degradation
0.39	3.24	PA4544 #	*rluD*	Ribosomal large subunit pseudouridine synthase D	Translation, post-translational modification, degradation
0.39	2.16	PA3808		Uncharacterized protein	Hypothetical, unclassified, unknown
0.39	2.06	PA0970 #	*tolR*	Protein TolR	Transport of small molecules
0.39	4.25	PA4005		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.39	2.88	PA4812	*fdnG*	Formate dehydrogenase-O, major subunit	Energy metabolism
0.38	3.12	PA5201		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.38	2.28	PA5556 #	*atpA*	ATP synthase subunit alpha	Energy metabolism
0.38	4.70	PA3496 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.38	4.43	PA0750 #	*ung*	Uracil-DNA glycosylase	DNA replication, recombination, modification & repair
0.38	5.96	PA0945	*purM*	Phosphoribosylformylglycinamidine cyclo-ligase	Nucleotide biosynthesis & metabolism
0.38	4.78	PA4006	*nadD*	Probable nicotinate-nucleotide adenylyltransferase	Biosynthesis of cofactors, prosthetic groups & carriers
0.37	2.90	PA1012		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.37	3.92	PA4004		UPF0247 protein PA4004	Hypothetical, unclassified, unknown
0.37	3.21	PA2023	*galU*	UTP--glucose-1-phosphate uridylyltransferase	Central intermediary metabolism
0.37	5.01	PA3980	*miaB*	UPF0004 protein PA3980	Hypothetical, unclassified, unknown
0.37	3.84	PA4640	*mqo2*	Probable malate:quinone oxidoreductase 2	Energy metabolism
0.37	3.73	PA5192	*pckA*	Phosphoenolpyruvate carboxykinase	Energy metabolism
0.37	8.06	PA5440		Probable peptidase	Putative enzymes
0.37	2.73	PA5568 #	*oxaA*	Inner membrane protein oxaA	Membrane proteins
0.36	4.43	PA0380		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.36	2.99	PA0429 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.36	3.98	PA4684		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.36	4.33	PA0767	*lepA*	GTP-binding protein LepA	Protein secretion & export apparatus
0.36	2.34	PA0705 #	*migA*	Probable glycosyl transferase	Cell wall, LPS & capsule
0.36	3.16	PA3169	*mtnA*	Probable methylthioribose-1-phosphate isomerase	Translation, post-translational modification, degradation
0.36	5.06	PA4853	*fis*	Putative fis-like DNA-binding protein	Transcriptional regulators
0.36	3.60	PA4268	*rpsL*	30S ribosomal protein S12	Translation, post-translational modification, degradation
0.35	6.77	PA4746		UPF0090 protein PA4746	Hypothetical, unclassified, unknown
0.35	2.82	PA5559 #	*atpE*	ATP synthase C chain	Energy metabolism
0.35	5.20	PA5561 #	*atpI*	ATP synthase protein I	Energy metabolism
0.35	2.38	PA5564 #	*gidB*	Methyltransferase GidB	Cell division
0.35	21.96	PA2840	*deaD*	Probable ATP-dependent RNA helicase	Transcription, RNA processing & degradation
0.35	3.16	PA4333	*fumA*	Probable fumarase	Energy metabolism
0.35	3.53	PA1475	*ccmA*	Cytochrome c biogenesis ATP-binding export protein CcmA	Transport of small molecules
0.35	4.17	PA2901		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.34	8.38	PA0654	*speD*	S-adenosylmethionine decarboxylase proenzyme	Amino acid biosynthesis & metabolism
0.34	5.08	PA3653	*frr*	Ribosome recycling factor	Translation, post-translational modification, degradation
0.34	3.30	PA2983		Probable TolQ-type transport protein	Transport of small molecules
0.34	4.36	PA4636		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	9.14	PA2971		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	4.46	PA5340		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	2.84	PA0968		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	2.32	PA2193 #	*hcnA*	Hydrogen cyanide synthase HcnA	Central intermediary metabolism
0.34	2.93	PA2740	*pheS*	Phenylalanyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation
0.34	4.56	PA4744	*infB*	Translation initiation factor IF-2	Translation, post-translational modification, degradation
0.34	4.72	PA4854	*purH*	Bifunctional purine biosynthesis protein PurH	Biosynthesis of cofactors, prosthetic groups & carriers
0.33	4.67	PA3654	*pyrH*	Uridylate kinase	Nucleotide biosynthesis & metabolism
0.33	3.18	PA5053	*hslV*	ATP-dependent protease HslV	Chaperones & heat shock proteins
0.33	4.41	PA2453		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.33	2.85	PA3645	*fabZ*	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase	Fatty acid & phospholipid metabolism
0.33	4.53	PA4747	*secG*	Protein-export membrane protein SecG	Protein secretion & export apparatus
0.32	2.43	PA2960 #	*pilZ*	PilZ protein	Motility & Attachment
0.32	4.21	PA3308	*rapA*	RNA polymerase-associated protein RapA	Transcription, RNA processing & degradation
0.32	9.21	PA5049	*rpmE*	50S ribosomal protein L31	Translation, post-translational modification, degradation
0.32	3.36	PA1192		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.32	2.25	PA3820 #	*secF*	Protein-export membrane protein SecF	Protein secretion & export apparatus
0.32	3.30	PA3821	*secD*	Protein-export membrane protein SecD	Protein secretion & export apparatus
0.31	2.30	PA1767 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.31	6.38	PA3824	*queA*	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	Translation, post-translational modification, degradation
0.31	4.30	PA5479	*gltP*	Proton-glutamate symporter	Transport of small molecules
0.31	2.53	PA3636	*kdsA*	2-dehydro-3-deoxyphosphooctonate aldolase	Cell wall, LPS & capsule
0.31	5.00	PA4052	*nusB*	N utilization substance protein B homolog	Transcription, RNA processing & degradation
0.31	4.59	PA3807	*ndk*	Nucleoside diphosphate kinase	Nucleotide biosynthesis & metabolism
0.31	2.22	PA4642 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.31	2.08	PA4664 #	*hemK*	Probable methyl transferase	Biosynthesis of cofactors, prosthetic groups & carriers
0.31	2.88	PA0888	*aotJ*	Arginine and ornithine binding protein	Transport of small molecules
0.30	2.04	PA1478	*ccmD*	Heme exporter protein D	Transport of small molecules
0.30	6.55	PA3823	*tgt*	Queuine tRNA-ribosyltransferase	Translation, post-translational modification, degradation
0.30	2.29	PA2739	*pheT*	Phenylalanyl-tRNA synthetase beta chain	Translation, post-translational modification, degradation
0.30	4.57	PA3701	*prfB*	Peptide chain release factor 2	Translation, post-translational modification, degradation
0.30	2.66	PA4933		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.30	5.60	PA4480	*mreC*	Rod shape-determining protein MreC	Cell division
0.30	4.55		tRNA_Leu		Translation, post-translational modification, degradation
0.29	6.24	PA4438		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.29	6.50	PA4852	*dusB*	tRNA-dihydrouridine synthase B	Translation, post-translational modification, degradation
0.29	6.55	PA0775		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.29	3.76	PA1674 #	*folE2*	GTP cyclohydrolase I 2	Biosynthesis of cofactors, prosthetic groups & carriers
0.29	9.01	PA1790		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.29	2.56	PA1812	*mltD*	Membrane-bound lytic murein transglycosylase D	Cell wall, LPS & capsule
0.28	2.45	PA3270 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.28	2.41	PA3811	*hscB*	Co-chaperone protein HscB homolog	Chaperones & heat shock proteins
0.28	6.41	PA3769	*guaA*	GMP synthase	Nucleotide biosynthesis & metabolism
0.28	3.54	PA1183	*dctA2*	C4-dicarboxylate transport protein 2	Transport of small molecules
0.28	3.40	PA4685		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.28	3.21	PA0009 #	*glyQ*	Glycyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation
0.28	5.98		tRNA_Asn		Translation, post-translational modification, degradation
0.28	10.47		tRNA_His		Translation, post-translational modification, degradation
0.28	4.06		tRNA_Val		Translation, post-translational modification, degradation
0.27	3.96	PA3245	*minE*	Cell division topological specificity factor	Cell division
0.27	2.78	PA4686 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.27	2.48	PA3313		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.27	4.84	PA3652	*uppS*	Undecaprenyl pyrophosphate synthetase	Cell wall, LPS & capsule
0.27	2.47	PA4770	*lldP*	L-lactate permease	Transport of small molecules

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.26	3.24	PA2798		Probable two-component response regulator	Two-component regulatory systems
0.26	5.47	PA2851	*efp*	Elongation factor P	Translation, post-translational modification, degradation
0.26	3.71	PA0760		UPF0350 protein PA0760	Hypothetical, unclassified, unknown
0.26	3.20	PA3162	*rpsA*	30S ribosomal protein S1	Translation, post-translational modification, degradation
0.25	2.56	PA3803 #	*ispG*	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.25	16.08	PA5286	*yjbQ*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.25	2.30	PA3804 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.25	2.32	PA0401 #	*pyrC*	Dihydroorotase-like protein	Nucleotide biosynthesis & metabolism
0.25	3.36	PA4031	*ppa*	Inorganic pyrophosphatase	Central intermediary metabolism
0.25	8.02	PA5117	*typA*	Regulatory protein TypA	Adaptation, Protection
0.24	8.29	PA4292		Probable phosphate transporter	Transport of small molecules
0.24	3.69	PA3472 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.24	2.99	PA4255	*rpmC*	50S ribosomal protein L29	Translation, post-translational modification, degradation
0.24	4.55	PA1554	*ccoN*	Probable cytochrome oxidase subunit	Energy metabolism
0.24	10.02	PA4743	*rbfA*	Ribosome-binding factor A	Translation, post-translational modification, degradation
0.23	4.79	PA3700	*lysS*	Lysyl-tRNA synthetase	Translation, post-translational modification, degradation
0.23	8.45	PA4432	*rpsI*	30S ribosomal protein S9	Translation, post-translational modification, degradation
0.23	4.14	PA3641		Probable amino acid permease	Transport of small molecules
0.23	13.59	PA3818	*suhB*	Inositol-1-monophosphatase	Translation, post-translational modification, degradation
0.23	9.89		tRNA_Ala		Translation, post-translational modification, degradation
0.22	2.94	PA0667		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.22	8.44	PA2970	*rpmF*	50S ribosomal protein L32	Translation, post-translational modification, degradation
0.22	5.57	PA4563	*rpsT*	30S ribosomal protein S20	Translation, post-translational modification, degradation
0.22	4.48	PA3806		UPF0063 protein PA3806	Hypothetical, unclassified, unknown
0.22	5.30		tRNA_Trp		Translation, post-translational modification, degradation
0.21	6.91	PA4569	*ispB*	Octaprenyl-diphosphate synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.21	10.50	PA5298	*xpt*	Xanthine phosphoribosyltransferase	Nucleotide biosynthesis & metabolism
0.21	7.48	PA5315	*rpmG*	50S ribosomal protein L33	Translation, post-translational modification, degradation
0.21	3.79	PA4673		Putative uncharacterized protein	Hypothetical, unclassified, unknown

FC wt + SHX vs. wt	FC Δd + SHX vs. wt + SHX	PA number	gene name	function of gene product	functional classification
0.20	8.67	PA4481	*mreB*	Rod shape-determining protein MreB	Cell division
0.20	8.28	PA5491		Probable cytochrome	Energy metabolism
0.20	4.84	PA5445	*psecoA*	Probable coenzyme A transferase	Putative enzymes
0.19	2.95	PA2638	*nuoB*	NADH-quinone oxidoreductase subunit B	Energy metabolism
0.19	3.34	PA4307	*pctC*	Chemotactic transducer PctC	Chemotaxis
0.18	8.05	PA3744	*rimM*	16S rRNA-processing protein RimM	Transcription, RNA processing & degradation
0.18	2.32	PA1837		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.18	5.89	PA4276	*secE*	Preprotein translocase subunit SecE	Protein secretion & export apparatus
0.18	10.00	PA4748	*tpiA*	Triosephosphate isomerase	Energy metabolism
0.17	2.44	PA4430		Probable cytochrome b	Energy metabolism
0.17	7.04	PA2629	*purB*	Adenylosuccinate lyase	Nucleotide biosynthesis & metabolism
0.16	8.44	PA4275	*nusG*	Transcription antitermination protein NusG	Transcription, RNA processing & degradation
0.16	4.60	PA5569 #	*rnpA*	Ribonuclease P protein component	Translation, post-translational modification, degradation
0.16	3.40	PA1838	*cysI*	Sulfite reductase	Central intermediary metabolism
0.16	7.10	PA4670	*prs*	Ribose-phosphate pyrophosphokinase	Nucleotide biosynthesis & metabolism
0.15	6.46	PA5560 #	*atpB*	ATP synthase a chain	Energy metabolism
0.15	11.51	PA3743	*trmD*	tRNA (guanine-N(1)-)-methyltransferase	Translation, post-translational modification, degradation
0.15	12.96	PA0578		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.15	6.86	PA4672	*pth*	Peptidyl-tRNA hydrolase	Translation, post-translational modification, degradation
0.15	17.17		tRNA_Lys		Translation, post-translational modification, degradation
0.13	10.63	PA0579	*rpsU*	30S ribosomal protein S21	Translation, post-translational modification, degradation
0.13	11.94	PA3745	*rpsP*	30S ribosomal protein S16	Translation, post-translational modification, degradation
0.13	11.55		tRNA_Gln		Translation, post-translational modification, degradation
0.10	18.11		tRNA_Ile		Translation, post-translational modification, degradation
0.07	18.08	PA2619	*infA*	Translation initiation factor IF-1	Translation, post-translational modification, degradation
0.06	10.59	PA5570 #	*rpmH*	50S ribosomal protein L34	Translation, post-translational modification, degradation
0.06	37.66		tRNA_Gly		Translation, post-translational modification, degradation

Appendix C

***Pseudomonas aeruginosa* PAO1 genes differentially regulated by ppGpp after 15 minutes of NaOH treatment of anaerobically grown mid-exponential phase planktonic cultures.** Experimental procedures and data analysis are described elsewhere (3.1.4.1). PA number, gene name, description of encoded proteins and functional classification are according to the "Pseudomonas Genome Database". Genes are sorted by their fold induction in wild type treated with NaOH ("wt + NaOH") compared to wild type ("wt"), fold changes of Δ*relA*Δ*spoT* mutant strain treated with NaOH ("Δr/Δs + NaOH") compared to wild type treated with NaOH ("wt + NaOH") are also indicated. Genes marked with an asterisk (*) were differentially regulated in Δ*relA*Δ*spoT* mutant strain treated with NaOH compared to Δ*relA*Δ*spoT* mutant strain, but regulation differed more than 2-fold from fold change expression observed in wild type treated with NaOH compared to wild type. Genes marked with a rhombus (#) were regulated only by ppGpp, but not by DksA in a similar experiment (Appendix D).

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
Class I – Induced by ppGpp and upregulated in response to NaOH treatment					
58.93	0.46	PA4739 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
33.97	0.26	PA3692 *		probable outer membrane protein precursor	Membrane proteins
33.33	0.35	PA3691 *		hypothetical protein	Hypothetical, unclassified, unknown
32.46	0.41	PA4738 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
24.55	0.21	PA1323 *		hypothetical protein	Hypothetical, unclassified, unknown
22.26	0.54	PA5481 *		hypothetical protein	Hypothetical, unclassified, unknown
22.25	0.18	PA1324 *		hypothetical protein	Hypothetical, unclassified, unknown
20.60	0.14	PA4880 *		probable bacterioferritin	Central intermediary metabolism
19.36	0.16	PA0567 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
17.71	0.17	PA0059 *	*osmC*	osmotically inducible protein OsmC	Adaptation & Protection
17.28	0.39	PA4876 *	*osmE*	osmotically inducible lipoprotein OsmE	Adaptation & Protection
12.23	0.47	PA2747 *		hypothetical protein	Hypothetical, unclassified, unknown
12.22	0.46	PA4714 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
10.84	0.19	PA0355	*pfpI*	protease PfpI	Translation, post-translational modification, degradation
10.81	0.47	PA2000 *		dehydrocarnitine CoA transferase, subunit B	Amino acid biosynthesis & metabolism
9.69	0.24	PA4877 *		hypothetical protein	Hypothetical, unclassified, unknown
7.21	0.54	PA0447 * #	*gcdH*	glutaryl-CoA dehydrogenase	Fatty acid & phospholipid metabolism

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
7.13	0.40	PA3795 *		probable oxidoreductase	Putative enzymes
6.79	0.31	PA2883 *		hypothetical protein	Hypothetical, unclassified, unknown
6.39	0.21	PA3042		hypothetical protein	Hypothetical, unclassified, unknown
5.91	0.32	PA1112		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.90	0.28	PA3040 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.76	0.41	PA2486 *		hypothetical protein	Hypothetical, unclassified, unknown
5.65	0.53	PA2433 *		hypothetical protein	Hypothetical, unclassified, unknown
5.51	0.24	PA3041 *		hypothetical protein	Hypothetical, unclassified, unknown
5.49	0.36	PA2166		hypothetical protein	Hypothetical, unclassified, unknown
5.43	0.29	PA0038		hypothetical protein	Hypothetical, unclassified, unknown
5.28	0.54	PA2001 *	*atoB*	acetyl-CoA acetyltransferase	Fatty acid & phospholipid metabolism
5.16	0.33	PA0586		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.00	0.08	PA0493		probable biotin-requiring enzyme	Putative enzymes
4.97	0.50	PA2485 * #		hypothetical protein	Hypothetical, unclassified, unknown
4.90	0.41	PA1562 *	*acnA*	aconitate hydratase 1	Energy metabolism
4.85	0.41	PA2553 *		probable acyl-CoA thiolase	Putative enzymes
4.71	0.43	PA4311		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.44	0.45	PA4345		hypothetical protein	Hypothetical, unclassified, unknown
4.35	0.32	PA0587		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.04	0.33	PA0745		probable enoyl-CoA hydratase/isomerase	Putative enzymes
4.02	0.33	PA0746		probable acyl-CoA dehydrogenase	Putative enzymes
3.82	0.18	PA3919		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.67	0.36	PA2190		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.62	0.44	PA2915 #		hypothetical protein	Hypothetical, unclassified, unknown
3.29	0.48	PA2557		Probable AMP-binding enzyme	Fatty acid & phospholipid metabolism
3.29	0.15	PA0494		Probable acyl-CoA carboxylase subunit	Putative enzymes
3.25	0.12	PA0495		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.03	0.30	PA1135	*hchA*	Chaperone protein hchA	Chaperones & heat shock proteins
3.01	0.36	PA3570	*mmsA*	Methylmalonate-semialdehyde dehydrogenase	Amino acid biosynthesis & metabolism

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
2.99	0.38	PA2754		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.91	0.43	PA2815		Probable acyl-CoA dehydrogenase	Putative enzymes
2.81	0.35	PA4803		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.68	0.35	PA0656		Probable HIT family protein	Putative enzymes
2.66	0.26	PA4607		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.62	0.45	PA0036 #	*trpB*	Tryptophan synthase beta chain	Amino acid biosynthesis & metabolism
2.62	0.29	PA0496		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.58	0.38	PA0232	*pcaC*	Gamma-carboxymuconolactone decarboxylase	Carbon compound catabolism
2.55	0.41	PA4793 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.26	PA3622	*rpoS*	RNA polymerase sigma factor RpoS	Transcriptional regulators
2.45	0.14	PA0492		UPF0271 protein PA0492	Hypothetical, unclassified, unknown
2.41	0.46	PA0744		Probable enoyl-CoA hydratase/isomerase	Putative enzymes
2.41	0.44	PA1984	*exaC1*	Probable aldehyde dehydrogenase	Putative enzymes
2.39	0.23	PA3161	*himD*	Integration host factor subunit beta	Transcriptional regulators
2.38	0.41	PA4674 #	*vapI*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.31	0.48	PA3857	*pcs*	Phosphatidylcholine synthase	Fatty acid & phospholipid metabolism
2.27	0.29	PA2796	*tal*	Transaldolase	Energy metabolism
2.25	0.38	PA4336		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.23	0.33	PA4015	*phaJ4*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.23	0.49	PA0055 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.20	0.38	PA0398		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.17	0.40	PA0747		Probable aldehyde dehydrogenase	Putative enzymes
2.13	0.35	PA5018	*msrA*	Peptide methionine sulfoxide reductase MsrA	Translation, post-translational modification, degradation
2.13	0.43	PA4026 #		Probable acetyltransferase	Putative enzymes
2.11	0.45	PA0558		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.11	0.47	PA1571 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.11	0.49	PA0026 #	*plcB*	Phospholipase C PlcB	Secreted factors (toxins, enzymes, alginate)
2.08	0.33	PA5191		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.07	0.49	PA0231	*pcaD*	Beta-ketoadipate enol-lactone hydrolase	Carbon compound catabolism

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
2.07	0.45	PA5271 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
Class II – Repressed by ppGpp and downregulated in response to NaOH treatment					
0.49			tRNA_Ala		Translation, post-translational modification, degradation
0.49	2.22	PA2994 #	*nqrF*	Na(+)-translocating NADH-quinone reductase subunit F	Energy metabolism
0.49	2.28	PA2453 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.49	2.51	PA5194		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.48	2.25	PA5296	*rep*	ATP-dependent DNA helicase Rep	DNA replication, recombination, modification & repair
0.48	2.64	PA1480 #	*ccmF*	Cytochrome c-type biogenesis protein CcmF	Energy metabolism
0.48	2.62	PA0009	*glyQ*	Glycyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation
0.47	2.51	PA1796	*folD*	Bifunctional protein FolD	Biosynthesis of cofactors, prosthetic groups & carriers
0.47	3.36	PA1687	*speE1*	Spermidine synthase 1	Central intermediary metabolism
0.46	2.33	PA1483	*cycH*	Cytochrome c-type biogenesis protein CycH	Amino acid biosynthesis & metabolism
0.46	2.71	PA1481	*ccmG*	cytochrome C biogenesis protein CcmG	Energy metabolism
0.46	2.28	PA1554 #	*ccoN*	Probable cytochrome oxidase subunit	Energy metabolism
0.46	2.16	PA4809	*fdhE*	Protein FdhE homolog	Energy metabolism
0.46	2.89	PA0760		UPF0350 protein PA0760	Hypothetical, unclassified, unknown
0.46	2.20	PA5335		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.45	3.32	PA5426	*purE*	Phosphoribosylaminoimidazole carboxylase catalytic subunit	Amino acid biosynthesis & metabolism
0.45	2.23	PA0548 #	*tktA*	Transketolase	Energy metabolism
0.45	2.51	PA1552		Probable cytochrome c	Energy metabolism
0.45	2.30	PA0382 #	*trmB*	tRNA (guanine-N(7)-)-methyltransferase	Translation, post-translational modification, degradation
0.44	2.10	PA4007	*proA*	Gamma-glutamyl phosphate reductase	Amino acid biosynthesis & metabolism
0.44	2.07	PA2627		UPF0274 protein PA2627	Hypothetical, unclassified, unknown
0.44	2.07	PA4684		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	2.85	PA5286		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	2.25	PA3763	*purL*	Phosphoribosylformylglycinamidine synthase	Nucleotide biosynthesis & metabolism
0.44	2.51	PA3980 #	*miaB*	UPF0004 protein PA3980	Translation, post-translational modification, degradation
0.44	2.16	PA4247	*rplR*	50S ribosomal protein L18	Translation, post-translational modification, degradation
0.43	2.13	PA4053	*ribH*	6,7-dimethyl-8-ribityllumazine synthase	Biosynthesis of cofactors, prosthetic groups & carriers

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.43	2.01	PA4479	*mreD*	Rod shape-determining protein MreD	Cell division
0.43	2.08	PA4480 #	*mreC*	Rod shape-determining protein MreC	Cell division
0.43	2.07	PA3652	*uppS*	Undecaprenyl pyrophosphate synthetase	Cell wall, LPS & capsule
0.43	2.25	PA4636		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.42	2.57	PA2996 #	*nqrD*	Na(+)-translocating NADH-quinone reductase	Energy metabolism
0.41	2.36	PA4481	*mreB*	Rod shape-determining protein MreB	Cell division
0.41	2.30	PA4234	*uvrA*	UvrABC system protein A	DNA replication, recombination, modification & repair
0.41	2.04	PA2630		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.41	2.01	PA3807	*ndk*	Nucleoside diphosphate kinase	Nucleotide biosynthesis & metabolism
0.41	2.25	PA4248	*rplF*	50S ribosomal protein L6	Translation, post-translational modification, degradation
0.41	2.19	PA4563	*rpsT*	30S ribosomal protein S20	Translation, post-translational modification, degradation
0.41	2.93	PA1478	*ccmD*	Heme exporter protein D	Transport of small molecules
0.41	2.31	PA5503	*metN2*	Methionine import ATP-binding protein MetN2	Transport of small molecules
0.40	2.64	PA1553 #	*fixO*	Probable cytochrome c oxidase subunit	Energy metabolism
0.40	2.55	PA0968		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	2.81	PA1837		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	2.14	PA4645	*hpt*	Probable purine/pyrimidine phosphoribosyl transferase	Nucleotide biosynthesis & metabolism
0.40	3.14	PA5298	*xpt*	Xanthine phosphoribosyltransferase	Nucleotide biosynthesis & metabolism
0.40	2.31	PA4276	*secE*	Preprotein translocase subunit SecE	Protein secretion/export apparatus
0.40	2.50	PA3169	*mtnA*	Probable methylthioribose-1-phosphate isomerase	Translation, post-translational modification, degradation
0.40	2.50	PA4482	*gatC*	Glutamyl-tRNA(Gln) amidotransferase subunit C	Translation, post-translational modification, degradation
0.40	3.43	PA5440		Probable peptidase	Translation, post-translational modification, degradation
0.39	2.71	PA1838	*cysI*	Sulfite reductase	Amino acid biosynthesis & metabolism
0.39	2.20	PA5491		Probable cytochrome	Energy metabolism
0.39	2.45	PA4686		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.39	2.13	PA5139		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.39	2.28	PA5336	*gmk*	Guanylate kinase	Nucleotide biosynthesis & metabolism
0.38	2.20	PA4748	*tpiA*	Triosephosphate isomerase	Energy metabolism
0.38	2.13	PA2740	*pheS*	Phenylalanyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.38	2.11	PA4239 #	*rpsD*	30S ribosomal protein S4	Translation, post-translational modification, degradation
0.38	2.14	PA4744	*infB*	Translation initiation factor IF-2	Translation, post-translational modification, degradation
0.37	2.10	PA4428 #	*sspA*	Stringent starvation protein A	Adaptation & Protection
0.37	2.83	PA5192	*pckA*	Phosphoenolpyruvate carboxykinase	Energy metabolism
0.37	2.81	PA1790		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.37	2.20	PA2744 #	*thrS*	Threonyl-tRNA synthetase	Translation, post-translational modification, degradation
0.37	2.23	PA4244	*rplO*	50S ribosomal protein L15	Translation, post-translational modification, degradation
0.36	2.35	PA3636 #	*kdsA*	2-dehydro-3-deoxyphosphooctonate aldolase	Cell wall, LPS & capsule
0.36	2.62	PA4333 #	*fumA*	Probable fumarase	Energy metabolism
0.36	3.12	PA3770	*guaB*	Inosine-5'-monophosphate dehydrogenase	Nucleotide biosynthesis & metabolism
0.36	2.11	PA0768	*lepB*	Signal peptidase I	Translation, post-translational modification, degradation
0.36	2.71	PA3655	*tsf*	Elongation factor Ts	Translation, post-translational modification, degradation
0.36	2.36	PA3823	*tgt*	Queuine tRNA-ribosyltransferase	Translation, post-translational modification, degradation
0.36	2.04	PA5565	*gidA*	glucose-inhibited division protein A	Cell division
0.35	2.16	PA3808 #		Uncharacterized protein	Hypothetical, unclassified, unknown
0.35	2.35	PA4745	*nusA*	N utilization substance protein A	Transcription, RNA processing & degradation
0.35	2.10	PA0767	*lepA*	GTP-binding protein LepA	Translation, post-translational modification, degradation
0.35	2.81	PA2851 #	*efp*	Elongation factor P	Translation, post-translational modification, degradation
0.35	2.30	PA3179	*rluB*	Ribosomal large subunit pseudouridine synthase B	Translation, post-translational modification, degradation
0.35	2.45	PA4432 #	*rpsI*	30S ribosomal protein S9	Translation, post-translational modification, degradation
0.35	2.22	PA4935	*rpsF*	30S ribosomal protein S6	Translation, post-translational modification, degradation
0.35	2.77	PA5504		Probable permease of ABC transporter	Transport of small molecules
0.35	2.25		tRNA_Gln		Translation, post-translational modification, degradation
0.34	3.39	PA1228		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	2.64	PA4685		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	3.16	PA0945	*purM*	Phosphoribosylformylglycinamidine cyclo-ligase	Nucleotide biosynthesis & metabolism
0.34	2.36	PA5049	*rpmE*	50S ribosomal protein L31	Translation, post-translational modification, degradation
0.33	2.69	PA3769	*guaA*	GMP synthase	Nucleotide biosynthesis & metabolism
0.33	2.64	PA2619	*infA*	Translation initiation factor IF-1	Translation, post-translational modification, degradation

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.33	4.20	PA4292		Probable phosphate transporter	Transport of small molecules
0.32	2.10	PA3809 #	*fdx*	2Fe-2S ferredoxin	Energy metabolism
0.32	2.73	PA0775		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.32	2.08	PA4243 #	*secY*	Preprotein translocase subunit SecY	Protein secretion/export apparatus
0.32	2.64	PA0579	*rpsU*	30S ribosomal protein S21	Translation, post-translational modification, degradation
0.32	2.41	PA3742	*rplS*	50S ribosomal protein L19	Translation, post-translational modification, degradation
0.31	2.10	PA4005		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.31	3.03	PA4275	*nusG*	Transcription antitermination protein NusG	Transcription, RNA processing & degradation
0.31	2.23	PA5570	*rpmH*	50S ribosomal protein L34	Translation, post-translational modification, degradation
0.31	2.30		tRNA_Ile		Translation, post-translational modification, degradation
0.30	2.87	PA2971		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.30	2.28	PA4853	*fis*	Putative fis-like DNA-binding protein	Transcriptional regulators
0.28	2.11	PA3811	*hscB*	Co-chaperone protein HscB homolog	Chaperones & heat shock proteins
0.28	2.30	PA1192		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.28	3.03	PA4670	*prs*	Ribose-phosphate pyrophosphokinase	Nucleotide biosynthesis & metabolism
0.28	2.62	PA2970	*rpmF*	50S ribosomal protein L32	Translation, post-translational modification, degradation
0.28	2.83	PA4743	*rbfA*	Ribosome-binding factor A	Translation, post-translational modification, degradation
0.27	2.25	PA4438		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.27	2.35	PA3308	*rapA*	RNA polymerase-associated protein RapA	Transcription, RNA processing & degradation
0.27	2.62	PA3745	*rpsP*	30S ribosomal protein S16	Translation, post-translational modification, degradation
0.26	2.77	PA0578		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.26	3.41	PA4673		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.26	3.58	PA3641		Probable amino acid permease	Transport of small molecules
0.25	2.17	PA5117	*typA*	Regulatory protein TypA	Adaptation & Protection
0.25	2.45	PA0654	*speD*	S-adenosylmethionine decarboxylase proenzyme	Central intermediary metabolism
0.25	2.07	PA4852	*dusB*	tRNA-dihydrouridine synthase B	Translation, post-translational modification, degradation
0.25	2.46	PA5315	*rpmG*	50S ribosomal protein L33	Translation, post-translational modification, degradation
0.25	2.58	PA5479 #	*gltP*	proton-glutamate symporter	Transport of small molecules
0.24	2.39	PA4933 #		hypothetical protein	Hypothetical, unclassified, unknown

FC wt + NaOH vs. wt	FC Δr/Δs + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.23	4.50	PA2629	*purB*	adenylosuccinate lyase	Amino acid biosynthesis & metabolism
0.23	2.27	PA3700 #	*lysS*	lysyl-tRNA synthetase	Translation, post-translational modification, degradation
0.23	2.89	PA3818		extragenic suppressor protein SuhB	Translation, post-translational modification, degradation
0.23	2.01	PA2638 * #	*nuoB*	NADH dehydrogenase I chain B	Energy metabolism
0.22	2.85	PA4255	*rpmC*	50S ribosomal protein L29	Translation, post-translational modification, degradation
0.22	3.43	PA4672		peptidyl-tRNA hydrolase	Translation, post-translational modification, degradation
0.19	3.32		tRNA_Gly		Translation, post-translational modification, degradation
0.17	2.68	PA3743 *	*trmD*	tRNA (guanine-N1)-methyltransferase	Translation, post-translational modification, degradation
Class III – Repressed by ppGpp, upregulated in response to NaOH treatment					
2.75	2.36	PA5367 * #	*pstA*	Membrane protein component of ABC phosphate transporter	Transport of small molecules
2.17	3.27	PA3781 * #		Probable transporter	Transport of small molecules
2.08	2.87	PA2386 * #	*pvdA*	L-ornithine 5-monooxygenase	Adaptation & Protection

Appendix D

***Pseudomonas aeruginosa* PAO1 genes differentially regulated by DksA after 15 minutes of NaOH treatment of anaerobically grown mid-exponential phase planktonic cultures.** Experimental procedures and data analysis are described elsewhere (3.1.4.1). PA number, gene name, description of encoded proteins and functional classification are according to the "*Pseudomonas* Genome Database". Genes are sorted by their fold induction in wild type treated with NaOH ("wt + NaOH") compared to wild type ("wt"), fold changes of Δ*dksA* mutant strain treated with NaOH ("Δd + NaOH") compared to wild type treated with NaOH ("wt + NaOH") are also indicated. Genes marked with an asterisk (*) were differentially regulated in Δ*dksA* mutant strain treated with NaOH compared to Δ*dksA* mutant strain, but regulation differed more than 2-fold from fold change expression observed in wild type treated with NaOH compared to wild type. Genes marked with a rhombus (#) were regulated only by DksA, but not by ppGpp in a similar experiment (Appendix C).

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
Class I – Induced by ppGpp in response to NaOH treatment					
58.93	0.07	PA4739 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
33.97	0.11	PA3692 *		probable outer membrane protein precursor	Cell wall, LPS & capsule
33.33	0.15	PA3691 *		hypothetical protein	Hypothetical, unclassified, unknown
32.46	0.07	PA4738 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
24.55	0.07	PA1323		hypothetical protein	Hypothetical, unclassified, unknown
22.26	0.09	PA5481		hypothetical protein	Hypothetical, unclassified, unknown
22.25	0.08	PA1324		hypothetical protein	Hypothetical, unclassified, unknown
20.60	0.05	PA4880		probable bacterioferritin	Hypothetical, unclassified, unknown
19.36	0.09	PA0567		conserved hypothetical protein	Hypothetical, unclassified, unknown
17.71	0.10	PA0059	*osmC*	osmotically inducible protein OsmC	Adaptation & Protection
17.28	0.21	PA4876 *	*osmE*	osmotically inducible lipoprotein OsmE	Adaptation & Protection
15.74	0.08	PA5482 * #		hypothetical protein	Hypothetical, unclassified, unknown
12.23	0.11	PA2747		hypothetical protein	Hypothetical, unclassified, unknown
12.22	0.44	PA4714 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
11.54	0.40	PA5212 * #		hypothetical protein	Hypothetical, unclassified, unknown
11.53	0.12	PA1999 * #		probable CoA transferase, subunit A	Carbon compound catabolism
10.84	0.12	PA0355	*pfpI*	protease PfpI	Translation, post-translational modification, degradation
10.81	0.13	PA2000		probable CoA transferase, subunit B	Carbon compound catabolism

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
10.62	0.17	PA2247 * #	*bkdA1*	2-oxoisovalerate dehydrogenase alpha subunit	Amino acid biosynthesis & metabolism
10.13	0.16	PA2249 #	*bkdB*	branched-chain alpha-keto acid dehydrogenase	Amino acid biosynthesis & metabolism
9.94	0.17	PA2250 #	*lpdV*	lipoamide dehydrogenase-Val	Energy metabolism
9.69	0.18	PA4877 *		hypothetical protein	Hypothetical, unclassified, unknown
8.47	0.18	PA2248 #	*bkdA2*	2-oxoisovalerate dehydrogenase beta subunit	Amino acid biosynthesis & metabolism
8.11	0.16	PA3369 #		hypothetical protein	Hypothetical, unclassified, unknown
7.37	0.24	PA0588 * #		conserved hypothetical protein	Hypothetical, unclassified, unknown
7.13	0.49	PA3795 *		probable oxidoreductase	Putative enzymes
6.79	0.32	PA2883		hypothetical protein	Hypothetical, unclassified, unknown
6.39	0.12	PA3042		hypothetical protein	Hypothetical, unclassified, unknown
5.91	0.39	PA1112 *		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.90	0.15	PA3040		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.76	0.36	PA2486 *		hypothetical protein	Hypothetical, unclassified, unknown
5.65	0.48	PA2433 *		hypothetical protein	Hypothetical, unclassified, unknown
5.51	0.09	PA3041		hypothetical protein	Hypothetical, unclassified, unknown
5.49	0.15	PA2166		hypothetical protein	Hypothetical, unclassified, unknown
5.43	0.34	PA0038		hypothetical protein	Hypothetical, unclassified, unknown
5.36	0.38	PA0865 #	*hpd*	4-hydroxyphenylpyruvate dioxygenase	Amino acid biosynthesis & metabolism
5.28	0.22	PA2001	*atoB*	acetyl-CoA acetyltransferase	Fatty acid & phospholipid metabolism
5.16	0.20	PA0586		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.08	0.37	PA2013 * #	*liuC*	putative 3-methylglutaconyl-CoA hydratase	Carbon compound catabolism
5.00	0.06	PA0493		probable biotin-requiring enzyme	Putative enzymes
4.90	0.33	PA1562	*acnA*	aconitate hydratase 1	Energy metabolism
4.85	0.17	PA2553		probable acyl-CoA thiolase	Putative enzymes
4.71	0.33	PA4311		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.44	0.30	PA4345		hypothetical protein	Hypothetical, unclassified, unknown
4.35	0.27	PA0587		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.30	0.33	PA2012 #	*liuD*	methylcrotonyl-CoA carboxylase, alpha-subunit	Carbon compound catabolism
4.06	0.32	PA1404 #		hypothetical protein	Hypothetical, unclassified, unknown

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
4.04	0.14	PA0745		probable enoyl-CoA hydratase/isomerase	Putative enzymes
4.02	0.18	PA0746		probable acyl-CoA dehydrogenase	Putative enzymes
3.82	0.20	PA3919		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.82	0.17	PA2381 #		hypothetical protein	Hypothetical, unclassified, unknown
3.73	0.43	PA0490 #		hypothetical protein	Hypothetical, unclassified, unknown
3.71	0.29	PA3370 #		hypothetical protein	Hypothetical, unclassified, unknown
3.67	0.28	PA2190		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.61	0.39	PA3889 #		probable binding protein component of ABC transporter	Transport of small molecules
3.34	0.26	PA3371 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.29	0.12	PA0494		Probable acyl-CoA carboxylase subunit	Putative enzymes
3.29	0.43	PA2557		Probable AMP-binding enzyme	Fatty acid & phospholipid metabolism
3.25	0.09	PA0495		Putative uncharacterized protein	Hypothetical, unclassified, unknown
3.16	0.34	PA2011 #	*luiE*	3-hydroxy-gamma-carboxygeranoyl-CoA lyase	Carbon compound catabolism
3.03	0.30	PA1135	*hchA*	Chaperone protein HchA	Chaperones & heat shock proteins
3.01	0.30	PA3570	*mmsA*	Methylmalonate-semialdehyde dehydrogenase	Amino acid biosynthesis & metabolism
2.99	0.26	PA2754		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.91	0.35	PA2554 #		Probable short-chain dehydrogenase	Putative enzymes
2.91	0.43	PA2815		Probable acyl-CoA dehydrogenase	Putative enzymes
2.85	0.30	PA2552 #	*acdB*	Probable acyl-CoA dehydrogenase	Putative enzymes
2.81	0.36	PA4803		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.77	0.27	PA5153 #		Probable periplasmic binding protein	Transport of small molecules
2.68	0.35	PA0656		Probable HIT family protein	Putative enzymes
2.68	0.31	PA4496 #		Probable binding protein component of ABC transporter	Transport of small molecules
2.66	0.28	PA4607		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.62	0.24	PA0496		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.58	0.26	PA0232	*pcaC*	Gamma-carboxymuconolactone decarboxylase	Carbon compound catabolism
2.58	0.41	PA1745 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.55	0.33	PA3622	*rpoS*	RNA polymerase sigma factor RpoS	Transcriptional regulators
2.55	0.42	PA5432 #		Probable acetyltransferase	Putative enzymes

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
2.45	0.09	PA0492		UPF0271 protein PA0492	Hypothetical, unclassified, unknown
2.41	0.31	PA0744		Probable enoyl-CoA hydratase/isomerase	Putative enzymes
2.41	0.17	PA1984	*exaC1*	Probable aldehyde dehydrogenase	Putative enzymes
2.41	0.46	PA3888 #		Probable permease of ABC transporter	Transport of small molecules
2.39	0.36	PA3161	*himD*	Integration host factor subunit beta	Transcriptional regulators
2.39	0.45	PA3890 #		Probable permease of ABC transporter	Transport of small molecules
2.35	0.45	PA3891 #		Probable ATP-binding component of ABC transporter	Transport of small molecules
2.31	0.41	PA3857	*pcs*	Phosphatidylcholine synthase	Fatty acid & phospholipid metabolism
2.27	0.36	PA2796	*tal*	Transaldolase	Energy metabolism
2.25	0.42	PA4336		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.23	0.41	PA4015	*phaJ4*	Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.20	0.37	PA0398		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.19	0.19	PA4502 #		Probable binding protein component of ABC transporter	Transport of small molecules
2.17	0.36	PA0747		Probable aldehyde dehydrogenase	Putative enzymes
2.17	0.39	PA5111 #	*gloA*	Lactoylglutathione lyase	Central intermediary metabolism
2.13	0.4	PA5018	*msrA*	Peptide methionine sulfoxide reductase MsrA	Translation, post-translational modification, degradation
2.11	0.49	PA0558		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.11	0.19	PA4501 #	*opdD*	Probable porin	Membrane proteins
2.10	0.36	PA1033 #		Probable glutathione S-transferase	Putative enzymes
2.08	0.45	PA5191		Putative uncharacterized protein	Hypothetical, unclassified, unknown
2.07	0.38	PA0231	*pcaD*	Beta-ketoadipate enol-lactone hydrolase	Carbon compound catabolism
Class II – Repressed by DksA in response to NaOH treatment					
0.49	2.38	PA2901 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.49	2.18	PA2999 #	*nqrA*	Na(+)-translocating NADH-quinone reductase subunit A	Energy metabolism
0.49	2.60	PA4519 #	*speC*	Ornithine decarboxylase	Amino acid biosynthesis & metabolism
0.49	2.08	PA4687 #	*hitA*	Ferric iron-binding periplasmic protein HitA	Transport of small molecules
0.49	2.09	PA4940 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.49	4.93	PA0277 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.49	2.25	PA3645 #	*fabZ*	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase	Fatty acid & phospholipid metabolism

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.49	2.87	PA5194		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.49	3.47		tRNA_Ala		Translation, post-translational modification, degradation
0.48	2.74	PA0009	*glyQ*	Glycyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation
0.48	2.08	PA3648 #		Probable outer membrane protein	Membrane proteins
0.48	2.95	PA4768 #	*smpB*	SsrA-binding protein	Translation, post-translational modification, degradation
0.48	2.62	PA5296	*rep*	ATP-dependent DNA helicase Rep	DNA replication, recombination, modification & repair
0.47	2.47	PA0592 #	*ksgA*	Dimethyladenosine transferase	Transcription, RNA processing & degradation
0.47	2.56	PA1796	*folD*	Bifunctional protein FolD	Biosynthesis of cofactors, prosthetic groups & carriers
0.47	2.02	PA4483 #	*gatA*	Glutamyl-tRNA(Gln) amidotransferase subunit A	Translation, post-translational modification, degradation
0.47	2.46	PA5316 #	*rpmB*	50S ribosomal protein L28	Translation, post-translational modification, degradation
0.47	4.46	PA1687	*speE1*	Spermidine synthase 1	Amino acid biosynthesis & metabolism
0.47	2.68	PA2626 #	*trmU*	Probable tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	Translation, post-translational modification, degradation
0.47	3.82	PA4928 #		UPF0313 protein PA4928	Hypothetical, unclassified, unknown
0.46	2.47	PA1481	*ccmG*	cytochrome C biogenesis protein CcmG	Energy metabolism
0.46	2.28	PA1483	*cycH*	Cytochrome c-type biogenesis protein CycH	Energy metabolism
0.46	2.25	PA4484 #	*gatB*	Aspartyl/glutamyl-tRNA amidotransferase subunit B	Translation, post-translational modification, degradation
0.46	3.92	PA5335		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.46	3.54	PA0760		UPF0350 protein PA0760	Hypothetical, unclassified, unknown
0.46	2.27	PA4809	*fdhE*	Protein fdhE homolog	Energy metabolism
0.46	2.87	PA3116 #		probable aspartate-semialdehyde dehydrogenase	Amino acid biosynthesis & metabolism
0.45	2.16	PA1552		Probable cytochrome c	Energy metabolism
0.45	2.34	PA3817 #		Probable methyltransferase	Putative enzymes
0.45	2.80	PA5048 #		Probable nuclease	Putative enzymes
0.45	3.05	PA5426	*purE*	Phosphoribosylaminoimidazole carboxylase catalytic subunit	Nucleotide biosynthesis & metabolism
0.44	2.53	PA3763	*purL*	Phosphoribosylformylglycinamidine synthase	Nucleotide biosynthesis & metabolism
0.44	2.76	PA4007	*proA*	Gamma-glutamyl phosphate reductase	Amino acid biosynthesis & metabolism
0.44	2.13	PA4666 #	*hemA*	Glutamyl-tRNA reductase	Translation, post-translational modification, degradation
0.44	2.02	PA2627		UPF0274 protein PA2627	Hypothetical, unclassified, unknown

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.44	2.20	PA4684		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	5.03	PA5286		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.44	2.29	PA0432 #	*sahH*	Adenosylhomocysteinase	Amino acid biosynthesis & metabolism
0.44	2.02	PA4247	*rplR*	50S ribosomal protein L18	Translation, post-translational modification, degradation
0.44	2.14	PA4268 #	*rpsL*	30S ribosomal protein S12	Translation, post-translational modification, degradation
0.43	2.50	PA0390 #	*metX*	Homoserine O-acetyltransferase	Amino acid biosynthesis & metabolism
0.43	2.29	PA4053	*ribH*	6,7-dimethyl-8-ribityllumazine synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.43	2.98	PA4636		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.43	2.34	PA3652	*uppS*	Undecaprenyl pyrophosphate synthetase	Cell wall, LPS & capsule
0.43	2.46	PA4479	*mreD*	Rod shape-determining protein MreD	Cell division
0.43	2.14	PA5239 #	*rho*	Transcription termination factor Rho	Transcription, RNA processing & degradation
0.42	2.02	PA4273 #	*rplA*	50S ribosomal protein L1	Translation, post-translational modification, degradation
0.42	2.45	PA4544 #	*rluD*	Ribosomal large subunit pseudouridine synthase D	Translation, post-translational modification, degradation
0.42	2.06	PA3637 #	*pyrG*	CTP synthase	Nucleotide biosynthesis & metabolism
0.41	2.03	PA4237 #	*rplQ*	50S ribosomal protein L17	Translation, post-translational modification, degradation
0.41	2.23	PA4563	*rpsT*	30S ribosomal protein S20	Translation, post-translational modification, degradation
0.41	2.86	PA5503	*metN2*	Methionine import ATP-binding protein MetN2	Transport of small molecules
0.41	2.08	PA2630		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.41	2.28	PA3807	*ndk*	Nucleoside diphosphate kinase	Nucleotide biosynthesis & metabolism
0.41	2.89	PA1478	*ccmD*	Heme exporter protein D	Transport of small molecules
0.41	2.09	PA2741 #	*rplT*	50S ribosomal protein L20	Translation, post-translational modification, degradation
0.41	2.15	PA4004 #		UPF0247 protein PA4004	Hypothetical, unclassified, unknown
0.41	2.12	PA4234	*uvrA*	UvrABC system protein A	DNA replication, recombination, modification & repair
0.41	2.45	PA4248	*rplF*	50S ribosomal protein L6	Translation, post-translational modification, degradation
0.41	3.30	PA0916 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.41	2.59	PA4481	*mreB*	Rod shape-determining protein MreB	Cell division
0.40	5.03	PA0968		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	2.04	PA3815 #		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	2.96	PA4482	*gatC*	Glutamyl-tRNA(Gln) amidotransferase subunit C	Translation, post-translational modification, degradation

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.40	3.28	PA4645	*hpt*	Probable purine/pyrimidine phosphoribosyl transferase	Nucleotide biosynthesis & metabolism
0.40	2.41	PA4264 #	*rpsJ*	30S ribosomal protein S10	Translation, post-translational modification, degradation
0.40	3.38	PA5298	*xpt*	Xanthine phosphoribosyltransferase	Nucleotide biosynthesis & metabolism
0.40	2.77	PA1837		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.40	2.90	PA3169	*mtnA*	Probable methylthioribose-1-phosphate isomerase	Translation, post-translational modification, degradation
0.40	2.02	PA4263 #	*rplC*	50S ribosomal protein L3	Translation, post-translational modification, degradation
0.40	2.01	PA4276	*secE*	Preprotein translocase subunit SecE	Protein secretion/export apparatus
0.40	6.92	PA5440		Probable peptidase	Putative enzymes
0.39	2.15	PA5139		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.39	3.65	PA5491		Probable cytochrome	Energy metabolism
0.39	2.83	PA3824 #	*queA*	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	Translation, post-translational modification, degradation
0.39	2.06	PA4686		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.39	3.82	PA5336	*gmk*	Guanylate kinase	Nucleotide biosynthesis & metabolism
0.39	2.18	PA1838	*cysI*	Sulfite reductase	Central intermediary metabolism
0.39	2.33	PA3806 #		UPF0063 protein PA3806	Hypothetical, unclassified, unknown
0.38	2.45	PA4006 #	*nadD*	Probable nicotinate-nucleotide adenylyltransferase	Biosynthesis of cofactors, prosthetic groups & carriers
0.38	2.34	PA4744	*infB*	Translation initiation factor IF-2	Translation, post-translational modification, degradation
0.38	2.10	PA2740	*pheS*	Phenylalanyl-tRNA synthetase alpha chain	Translation, post-translational modification, degradation
0.38	2.95	PA4748	*tpiA*	Triosephosphate isomerase	Energy metabolism
0.37	4.11	PA1790		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.37	2.01	PA4244	*rplO*	50S ribosomal protein L15	Translation, post-translational modification, degradation
0.37	2.06	PA4665 #	*prfA*	Peptide chain release factor 1	Translation, post-translational modification, degradation
0.37	2.91	PA5192	*pckA*	Phosphoenolpyruvate carboxykinase	Energy metabolism
0.36	2.53	PA5118 #	*thiI*	Thiamine biosynthesis protein ThiI	Biosynthesis of cofactors, prosthetic groups & carriers
0.36	3.03	PA3823	*tgt*	Queuine tRNA-ribosyltransferase	Translation, post-translational modification, degradation
0.36	2.50	PA0768	*lepB*	Signal peptidase I	Protein secretion/export apparatus
0.36	4.09	PA3655	*tsf*	Elongation factor Ts	Translation, post-translational modification, degradation
0.36	3.72	PA3770	*guaB*	Inosine-5'-monophosphate dehydrogenase	Nucleotide biosynthesis & metabolism
0.36	2.80	PA5565	*gidA*	glucose-inhibited division protein A	Cell division

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.35	2.95	PA4745	*nusA*	N utilization substance protein A	Transcription, RNA processing & degradation
0.35	3.39	PA5504		Probable permease of ABC transporter	Transport of small molecules
0.35	3.41	PA0767	*lepA*	GTP-binding protein LepA	Protein secretion/export apparatus
0.35	2.86	PA4935	*rpsF*	30S ribosomal protein S6	Translation, post-translational modification, degradation
0.35	7.39	PA3179	*rluB*	Ribosomal large subunit pseudouridine synthase B	Translation, post-translational modification, degradation
0.35	3.18		tRNA_Gln		Translation, post-translational modification, degradation
0.34	3.67	PA0945	*purM*	Phosphoribosylformylglycinamidine cyclo-ligase	Nucleotide biosynthesis & metabolism
0.34	2.64	PA4685		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	7.62	PA1228		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.34	6.07	PA5049	*rpmE*	50S ribosomal protein L31	Translation, post-translational modification, degradation
0.33	2.04	PA4854 #	*purH*	Bifunctional purine biosynthesis protein PurH	Nucleotide biosynthesis & metabolism
0.33	2.17	PA5193 #	*hslO*	33 kDa chaperonin	Chaperones & heat shock proteins
0.33	5.22	PA4292		Probable phosphate transporter	Transport of small molecules
0.33	5.22	PA2619	*infA*	Translation initiation factor IF-1	Translation, post-translational modification, degradation
0.33	5.43	PA2840 #	*deaD*	Probable ATP-dependent RNA helicase	Transcription, RNA processing & degradation
0.33	3.23	PA3769	*guaA*	GMP synthase	Nucleotide biosynthesis & metabolism
0.33	2.15	PA4307 #	*pctC*	Chemotactic transducer PctC	Chemotaxis
0.33	2.30	PA5564 #	*gidB*	Methyltransferase GidB	Cell division
0.32	3.76	PA0579	*rpsU*	30S ribosomal protein S21	Translation, post-translational modification, degradation
0.32	6.16	PA0775		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.32	2.70	PA3742	*rplS*	50S ribosomal protein L19	Translation, post-translational modification, degradation
0.32	2.14	PA4742 #	*truB*	tRNA pseudouridine synthase B	Translation, post-translational modification, degradation
0.32	3.68	PA4753 #		Probable RNA-binding protein	Hypothetical, unclassified, unknown
0.32	2.89	PA4746 #		UPF0090 protein PA4746	Hypothetical, unclassified, unknown
0.31	2.14	PA5570	*rpmH*	50S ribosomal protein L34	Translation, post-translational modification, degradation
0.31	3.57	PA4005		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.31	2.89	PA4275	*nusG*	Transcription antitermination protein NusG	Transcription, RNA processing & degradation
0.31	4.06		tRNA_Ile		Translation, post-translational modification, degradation
0.30	2.34	PA3701 #	*prfB*	Peptide chain release factor 2	Translation, post-translational modification, degradation

FC wt + NaOH vs. wt	FC Δd + NaOH vs. wt + NaOH	PA number	gene name	function of gene product	functional classification
0.30	3.01	PA4853	*fis*	Putative fis-like DNA-binding protein	Transcriptional regulators
0.30	4.68	PA2971		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.30	2.35	PA4741 #	*rpsO*	30S ribosomal protein S15	Translation, post-translational modification, degradation
0.28	2.45	PA3744 #	*rimM*	16S rRNA-processing protein RimM	Translation, post-translational modification, degradation
0.28	3.25	PA4670	*prs*	Ribose-phosphate pyrophosphokinase	Nucleotide biosynthesis & metabolism
0.28	2.45	PA4743	*rbfA*	Ribosome-binding factor A	Translation, post-translational modification, degradation
0.28	2.03	PA3811	*hscB*	Co-chaperone protein HscB homolog	Chaperones & heat shock proteins
0.28	2.52	PA1192		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.28	3.66	PA2970	*rpmF*	50S ribosomal protein L32	Translation, post-translational modification, degradation
0.27	2.72	PA3308	*rapA*	RNA polymerase-associated protein RapA	Transcription, RNA processing & degradation
0.27	3.12	PA3745	*rpsP*	30S ribosomal protein S16	Translation, post-translational modification, degradation
0.27	4.13	PA4438		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.26	3.67	PA4673		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.26	4.24	PA0578		Putative uncharacterized protein	Hypothetical, unclassified, unknown
0.26	2.14	PA3641		Probable amino acid permease	Transport of small molecules
0.25	3.13	PA5117	*typA*	Regulatory protein TypA	Adaptation & Protection
0.25	3.53	PA4852	*dusB*	tRNA-dihydrouridine synthase B	Translation, post-translational modification, degradation
0.25	3.43	PA5315	*rpmG*	50S ribosomal protein L33	Translation, post-translational modification, degradation
0.25	5.14	PA0654	*speD*	S-adenosylmethionine decarboxylase proenzyme	Amino acid biosynthesis & metabolism
0.23	4.59	PA2629	*purB*	adenylosuccinate lyase	Nucleotide biosynthesis & metabolism
0.23	4.42	PA3818		extragenic suppressor protein SuhB	Translation, post-translational modification, degradation
0.22	2.74	PA4255	*rpmC*	50S ribosomal protein L29	Translation, post-translational modification, degradation
0.22	4.97	PA4672		peptidyl-tRNA hydrolase	Translation, post-translational modification, degradation
0.19	6.48		tRNA_Gly		Translation, post-translational modification, degradation
0.17	4.24	PA3743 #	*trmD*	tRNA (guanine-N1)-methyltransferase	Translation, post-translational modification, degradation

Appendix E

Previously not annotated, putative *Pseudomonas putida* KT2440 ORF's identified by *in silico* analysis with "GLIMMER3" and subsequent BLASTP analysis. Results and e-values for BLASTP analysis were obtained from NCBI, details on data analysis are described elsewhere (2.10.2). "start"/"end" refer to the genomic position of identified genes, indicated PP numbers were appointed for annotation in the "Wikiputida" database. Genes marked with asterisks (*) were not given the appendix ".1" as they are located between two previously annotated genes with non-consecutive numbers.

appointed PP number	start	end	length (bp)	BLASTP analysis (best scored protein)	BLASTP analysis (best scored organism)	e-value
PP0004.1	4857	4705	152	hypothetical protein Pput_5307	*P. putida* F1	2,00E-016
PP0022.1	27288	27455	167	hypothetical protein Pput_0038	*P. putida* F1	7,00E-024
PP0134.1	143052	143342	290	hypothetical protein Pput_0118	*P. putida* F1	4,00E-119
PP0168.1	220688	221029	341	hypothetical protein Pput_0189	*P. putida* F1	2,00E-058
PP0201.1	251820	252062	242	hypothetical protein Pput_0223	*P. putida* F1	1,00E-037
PP0201.2	252168	252770	602	hypothetical protein Pput_0224	*P. putida* F1	1,00E-112
PP0273.1	333085	333213	128	hypothetical protein Pput_0290	*P. putida* F1	5,00E-017
PP0284.1	343442	342999	443	hypothetical protein Pput_0303	*P. putida* F1	5,00E-080
PP0305.1	367312	366695	617	hypothetical protein Pput_0326	*P. putida* F1	5,00E-047
PP0348.1	424154	424014	140	hypothetical protein Pput_0374	*P. putida* F1	8,00E-014
PP0373.1	454254	453733	521	hypothetical protein StAA4_09640	*Streptomyces sp.* AA4	9,00E-007
PP0628.1	736839	736390	449	hypothetical protein Pput_0669	*P. putida* F1	3,00E-070
PP0651.1	759860	760087	227	hypothetical protein Pput_0684	*P. putida* F1	5,00E-036
PP0655.1	763276	763635	359	hypothetical protein Pput_0689	*P. putida* F1	1,00E-059
PP0757.1	873365	873625	260	hypothetical protein Pput_0785	*P. putida* F1	1,00E-039
PP0781.1	898501	898686	185	hypothetical protein Pput_0805	*P. putida* F1	3,00E-016
PP0799.1	919527	919303	224	hypothetical protein Pput_0823	*P. putida* F1	2,00E-022
PP0820.1	959194	959733	539	hypothetical protein Pput_0847	*P. putida* F1	1,00E-095
PP1115.1	1275412	1275113	299	hypothetical protein PP_2294	*P. putida* F1	3,00E-036
PP1411.1	1610473	1610922	449	hypothetical protein Pput_4310	*P. putida* F1	3,00E-067
PP1537.1	1741024	1740902	122	hypothetical protein PputGB1_3466	*P. putida* GB-1	2,00E-009

appointed PP number	start	end	length (bp)	BLASTP analysis (best scored protein)	BLASTP analysis (best scored organism)	e-value
PP1546.1	1747402	1747286	116	LuxR family DNA binding protein	*P. entomophila* L48	5,00E-008
PP1548.1	1749022	1749315	293	hypothetical protein Pput_4132	*P. putida* F1	8,00E-015
PP1578.1	1768872	1769252	380	conserved hypothetical protein	*Klebsiella pneumoniae* ATCC13884	4,00E-027
PP1781.1	1995092	1994979	113	hypothetical protein Pput_1376	*P. putida* F1	1,00E-010
PP1810.1	2037877	2037602	275	hypothetical protein Pput_3899	*P. putida* F1	6,00E-045
PP1923.1	2171190	2171462	272	hypothetical protein Psyrpo1_17278	*P. syringae pv. oryzae*	2,00E-006
PP1935.1	2182364	2182579	215	hypothetical protein PputGB1_1620	*P. putida* GB-1	2,00E-017
PP1935.2	2183476	2183766	290	hypothetical protein PputGB1_1622	*P. putida* GB-1	7,00E-031
PP2025.1	2301996	2302337	341	hypothetical protein PputGB1_1558	*P. putida* GB-1	2,00E-006
PP2044.1	2325017	2325310	293	hypothetical protein Pput_3697	*P. putida* F1	3,00E-036
PP2095.1	2388619	2388404	216	ribosome modulation factor	*P. putida* F1	9,00E-035
PP2127.1	2427724	2427212	512	putative CheA signal transduction histidine kinase	*P. putida* F1	7,00E-086
PP2207.1	2514537	2514421	116	diguanylate phosphodiesterase	*P. putida* F1	2,00E-008
PP2238.1	2547119	2546838	281	lipoprotein	*P. putida* GB-1	7,00E-047
PP2284.1	2607889	2608302	413	hypothetical protein epsilon15p13	*Enterobacteria* epsilon15	4,00E-015
PP2294.1	2622611	2622808	197	unknown	*Delftia tsuruhatensis*	2,00E-023
PP2437.1	2785058	2785207	149	hypothetical protein PputGB1_2083	*P. putida* GB-1	6,00E-010
PP2444.1	2792635	2792907	272	hypothetical protein Pput_3249	*P. putida* F1	9,00E-042
PP2476.1	2823673	2823822	149	conserved hypothetical protein	*Acinetobacter sp.* RUH2624	2,00E-009
PP2507.1	2855382	2855119	263	hypothetical protein Pput_3212	*P. putida* F1	4,00E-044
PP2507.2	2855666	2855532	134	hypothetical protein Pput_3211	*P. putida* F1	9,00E-016
PP2508.1	2856328	2856567	239	hypothetical protein PputGB1_3425	*P. putida* GB-1	2,00E-014
PP2512.1	2859761	2860219	458	hypothetical protein Pput_3206	*P. putida* F1	8,00E-074
PP2521 *	2868537	2869313	776	glutaminase	*P. putida* F1	5,00E-144
PP2541.1	2886438	2886109	329	YD repeat protein	*P. syringae pv. tomato* DC3000	4,00E-004
PP2653.1	3042095	3042409	314	hypothetical protein PputGB1_3151	*P. putida* GB-1	3,00E-041
PP2873.1	3275654	3275842	188	hypothetical protein PputGB1_2908	*P. putida* GB-1	6,00E-029
PP3014.1	3404158	3403982	176	hypothetical protein Pput_2678	*P. putida* F1	3,00E-026
PP3032.1	3419172	3418861	311	hypothetical protein Pmen_3985	*P. mendocina* ymp	2,00E-005

appointed PP number	start	end	length (bp)	BLASTP analysis (best scored protein)	BLASTP analysis (best scored organism)	e-value
PP3036.1	3421121	3421639	518	hypothetical protein PSPTO_3420	*P. syringae pv. tomato* DC3000	7,00E-077
PP3066.1	3447232	3447792	560	phage protein, putative	*P. putida* GB-1	3,00E-058
PP3066.2	3448129	3447872	257	unknown	*Picea sitchensis*	4,00E+004
PP3081.1	3467254	3466946	308	hypothetical protein Pput_2642	*P. putida* F1	4,00E-047
PP3090.1	3482120	3481212	908	hypothetical protein Pput_2632	*P. putida* F1	3,00E-172
PP3100.1	3497593	3496829	764	hypothetical protein Pput_2621	*P. putida* F1	2,00E-144
PP3100.2	3498784	3498299	485	hypothetical protein Pput_2620	*P. putida* F1	2,00E-089
PP3101.1	3500322	3500050	272	hypothetical protein PFL_6098	*P. fluorescens* Pf-5	3,00E-024
PP3101.2	3500676	3500539	137	hypothetical protein MUL_2318	*Mycobacterium ulcerans* Agy99	4,00E-006
PP3101.3	3501234	3501455	221	Rhs element Vgr protein	*P. putida* F1	4,00E-028
PP3101.4	3502106	3502309	203	hypothetical protein PP_3105	*P. putida* KT2440	2,00E-011
PP3103.1	3506310	3506119	191	hypothetical protein PputW619_2515	*P. putida* W619	6,00E-020
PP3106.1	3510237	3510821	584	hypothetical protein Pput_2617	*P. putida* F1	1,00E-104
PP3108.1	3516127	3516246	119	YD repeat-containing protein	*P. putida* F1	9,00E-008
PP3108.2	3516293	3516916	623	YD repeat-containing protein	*P. putida* F1	6,00E-109
PP3115.1	3525451	3525188	263	HAD family hydrolase	*P. putida* F1	3,00E-042
PP3125.1	3537180	3537329	149	hypothetical protein PputGB1_2731	*P. putida* GB-1	3,00E-019
PP3126.1	3538266	3539291	1025	hypothetical protein Pput_2589	*P. putida* F1	0,00E+000
PP3200.1	3631104	3632471	1367	hypothetical protein AZL_c03810	*Azospirillum sp.* B510	1,00E-144
PP3203.1	3637257	3637421	164	short-chain dehydrogenase/reductase SDR	*Comamonas testosteroni* KF-1	0,00E+000
PP3233.1	3671129	3670404	725	hypothetical protein Pput_2461	*P. putida* F1	2,00E-120
PP3240.1	3676333	3676082	251	hypothetical protein Pput_2521	*P. putida* F1	1,00E-139
PP3250.1	3685124	3685315	191	hypothetical protein Pput_2511	*P. putida* F1	4,00E-025
PP3262.1	3697485	3697069	416	hypothetical protein Pput_2498	*P. putida* F1	5,00E-075
PP3269.1	3703174	3703443	269	hypothetical protein Pput_2490	*P. putida* F1	1,00E-045
PP3328.1	3765667	3766539	872	LysR family transcriptional regulator	*P. putida* F1	6,00E-156
PP3329.1	3767983	3768372	389	hypothetical protein Pput_2413	*P. putida* F1	2,00E-066
PP3333.1	3773295	3774302	1007	Possible TonB-dependent receptor	*Ralstonia solanacearum* UW551	2,00E-063
PP3464.1	3931104	3929812	1292	FAD dependent oxidoreductase	*P. putida* F1	0,00E+000

appointed PP number	start	end	length (bp)	BLASTP analysis (best scored protein)	BLASTP analysis (best scored organism)	e-value
PP3470 *	3937316	3937696	380	transposase, IS4 family protein	*P. putida* F1	3,00E-060
PP3490.1	3960748	3960473	275	hypothetical protein VF_A1080	*Vibrio fischeri* ES114	4,00E+004
PP3603.1	4095665	4096315	650	hypothetical protein Pput_3103	*P. putida* F1	8,00E-110
PP3644.1	4140369	4140965	596	hypothetical protein Pput_2086	*P. putida* F1	2,00E-111
PP3673 *	4174337	4174023	314	transposase IS4 family protein	*P. putida* W619	1,00E-040
PP3684.1	4189319	4189552	233	transcriptional regulator	*Marinomonas sp.* MWYL1	4,00E-010
PP3687 *	4192703	4192963	260	ISPpu14, transposase Orf3	*P. putida* KT2440	3,00E-039
PP3697.1	4217397	4217035	362	hypothetical protein CJA_1586	*Cellvibrio japonicus* Ueda107	3,00E-026
PP3709.1	4232110	4231820	290	hypothetical protein PsyrptA_25925	*P. syringae pv. tabaci* ATCC11528	6,00E-026
PP3812.1	4338813	4338520	293	hypothetical protein Pput_1957	*P. putida* F1	6,00E-050
PP3850.1	4375425	4375276	149	hypothetical protein Pput_3355	*P. putida* F1	1,00E-020
PP3913.1	4422945	4422646	299	hypothetical protein Pput_1372	*P. putida* F1	4,00E-033
PP3914.1	4423650	4423769	119	hypothetical protein Pput_4144	*P. putida* F1	2,00E-008
PP3927.1	4433442	4433092	350	hypothetical protein Pput_1907	*P. putida* F1	4,00E-062
PP3987.1	4497948	4496701	1247	hypothetical protein RSc3437	*Ralstonia solanacearum* GMI1000	4,00E-032
PP4023 *	4535546	4536433	887	hypothetical protein Pput_1810	*P. putida* F1	2,00E-112
PP4090 *	4625028	4625936	908	RHS family protein, putative	*P. putida* KT2440	1,00E-167
PP4194.1	4741161	4740877	284	lipid-binding START domain-containing protein	*P. putida* F1	2,00E-037
PP4270.1	4856498	4856223	275	hypothetical protein Pput_1597	*P. putida* F1	3,00E-046
PP4407.1	5001143	5001268	125	ISPs1, transposase OrfA	*P. putida* W619	7,00E-014
PP4407.2	5001354	5001767	413	transposase IS4 family protein	*P. putida* W619	4,00E-067
PP4415.1	5012594	5012418	176	hypothetical protein PsyrptA_26155	*P. syringae pv. tabaci* ATCC11528	4,00E-012
PP4420.1	5015735	5015523	212	DNA-directed DNA polymerase	*P. putida* F1	8,00E-028
PP4450.1	5047632	5047465	167	hypothetical protein Pput_5027	*P. putida* F1	1,00E-012
PP4467.1	5074035	5073673	362	hypothetical protein PFLU2478	*P. fluorescens* SBW25	5,00E-028
PP4491.1	5103256	5103384	128	hypothetical protein Pput_1422	*P. putida* F1	3,00E-015
PP4533.1	5148537	5148268	269	hypothetical protein Pput_1376	*P. putida* F1	2,00E-045
PP4538.1	5155464	5155177	287	hypothetical protein Pput_1352	*P. putida* F1	8,00E-049
PP4583.1	5204061	5203783	278	hypothetical protein PputGB1_4106	*P. putida* GB-1	7,00E-020

appointed PP number	start	end	length (bp)	BLASTP analysis (best scored protein)	BLASTP analysis (best scored organism)	e-value
PP4613.1	5237168	5237515	347	hypothetical protein Pput_4471	*P. putida* F1	4,00E-062
PP4629.1	5253088	5253735	647	hypothetical protein Pput_4490	*P. putida* F1	8,00E-113
PP4633.1	5256505	5256846	341	HopJ type III effector protein	*P. putida* F1	3,00E-058
PP4694.1	5333409	5333585	176	hypothetical protein PSEEN4728	*P. entomophila* L48	4,00E-024
PP4739.1	5391082	5391345	263	hypothetical protein Pput_3212	*P. putida* F1	1,00E-038
PP4739.2	5391606	5391409	197	hypothetical protein P3TCK_11088	*Photobacterium profundum* 3TCK	1,00E-012
PP4739.3	5391875	5391624	251	hypothetical protein P3TCK_11088	*Photobacterium profundum* 3TCK	3,00E-005
PP4742.1	5398728	5399156	428	hypothetical protein Dde_2495	*Desulfovibrio desulfuricans subsp. desulfuricans* G20	2,00E-045
PP4763.1	5423617	5424303	686	putative phosphohistidine phosphatase SixA	*P. putida* F1	2,00E-128
PP4771.1	5432124	5432663	539	hypothetical protein Pput_4647	*P. putida* F1	6,00E-099
PP4825.1	5490779	5490123	656	hypothetical protein Pput_4701	*P. putida* F1	1,00E-016
PP4825.2	5491461	5490772	689	putative transcriptional regulator	*Streptococcus dysgalactiae* GGS_124	0,00E+000
PP4825.3	5492196	5491513	683	hypothetical protein PSEEN4867	*P. entomophila* L48	2,00E-016
PP4884 *	5552804	5552953	149	ISPpu9, transposase	*P. putida* KT2440	3,00E-015
PP4947.1	5635044	5634802	242	hypothetical protein Pput_4820	*P. putida* F1	9,00E-035
PP5062.1	5773429	5774085	656	transcriptional regulator BetI	*P. putida* F1	1,00E-121
PP5129.1	5852332	5852619	287	DNA gyrase, A subunit	*Burkholderia multivorans* CGD2M	4,00E+004
PP5201.1	5934113	5934331	218	hypothetical protein Pput_5109	*P. putida* F1	2,00E-031
PP5235.1	5968854	5969207	353	hypothetical protein Pput_5145	*P. putida* F1	3,00E-060
PP5266.1	6016675	6015833	842	hypothetical protein PP_4242	*P. putida* KT2440	4,00E-028
PP5395.1	6152004	6152393	389	hypothetical protein Psyrpo1_22981	*P. syringae pv. oryzae* 1_6	4,00E-062

Appendix F

***Pseudomonas putida* KT2440 genes with putative Anr-dependent promoters identified by „Virtual Footprint – Regulon Analysis“ with a single pattern search for position weight matrix „Anr_Dnr“ of *Pseudomonas aeruginosa* PAO1.** Genes are sorted by PP number, “start” / “end” describe the genomic position of putative Anr box, “strand” the orientation of Anr box, “ATG distance” distance of Anr box to the translational start of indicated gene and “PMW” score the position weight matrix score of the predicted Anr box. Description of encoded gene products is according to “*Pseudomonas* Genome Database”. Note that only *P. aeruginosa* orthologs with a predicted Anr binding site in the promoter are listed.

PP number	gene name	start	end	strand	ATG distance	PWM Score	sequence of predicted Anr box	function of gene product	*P. aeruginosa* orthologs with predicted Anr box in promoter	
									PA number	gene name
PP0028		32327	32340	-	124	11.83	TTGTCTCATTTCAA	Putative uncharacterized protein		
PP0046		52369	52382	+	12	12.29	CTGCCGTGAATCAA	Porin, putative		
PP0068	*def-1*	79143	79156	-	104	11.54	TTGGGGGCTATCAA	peptide deformylase		
PP0069		79143	79156	-	90	11.54	TTGGGGGCTATCAA	Smf protein		
PP0072	*qor-1*	83181	83194	+	53	11.77	TTGGTCGTCGTCAA	Quinone oxidoreductase	PA0023	*qor*
PP0073	*hemF*	83181	83194	+	67	11.77	TTGGTCGTCGTCAA	Coproporphyrinogen-III oxidase, aerobic	PA0024	*hemF*
PP0127		132436	132449	+	128	12.25	ATGTTGTAGGTCAA	Thiol:disulfide interchange protein, DsbA family		
PP0137	*gltP*	144495	144508	+	258	12.52	ATGTTATAAATCAA	Proton/sodium-glutamate/aspartate symporter		
PP0154		163937	163950	-	105	14.35	TTGATCCGGATCAA	Acetyl-CoA hydrolase/transferase family protein		
PP0175		226686	226699	-	26	12.68	TTGCTAACTATCAA	Transcriptional regulator, MarR family		
PP0180		231799	231812	-	89	12.28	TTGCTACAAATCAG	Cytochrome c family protein		
PP0182		234565	234578	+	74	11.70	ATGACGCTGGTCAG	Putative uncharacterized protein		
PP0183		234565	234578	+	62	11.70	ATGACGCTGGTCAG	Glutathione S-transferase family protein		
PP0202		253311	253324	-	97	11.74	ATGACGTTTTTCAA	CBS domain protein	PA0250	
PP0273		332737	332750	+	53	14.06	TTGACGGGCATCAA	Putative uncharacterized protein	PA0200	
PP0403	*surA*	489778	489791	-	162	11.87	CTGCGCCTGATCAA	Chaperone surA		
PP0431		518591	518604	-	124	13.12	TTGCCCCTTATCAA	Putative uncharacterized protein		
PP0432	*argCI*	518591	518604	-	6	13.12	TTGCCCCTTATCAA	N-acetyl-gamma-glutamyl-phosphate reductase 1		
PP0449	*rpsL*	546048	546061	-	109	11.65	TTGCACCCAGTCAA	30S ribosomal protein S12		
PP0481	*katA*	564419	564432	-	87	13.24	ATGACTTACATCAA	Catalase		

PP number	gene name	start	end	strand	ATG distance	PWM Score	sequence of predicted Anr box	function of gene product	*P. aeruginosa* orthologs with predicted Anr box in promoter	
									PA number	gene name
PP0486		573147	573160	-	60	12.38	TTGAGTCGATTCAA	Transcriptional regulator, GntR family		
PP0487		573147	573160	-	74	12.38	TTGAGTCGATTCAA	Membrane protein, putative		
PP0488		574036	574049	-	4	13.21	TTGTCTCCTATCAA	Oxidoreductase, short chain dehydrogenase/ reductase family		
PP0504	*oprG*	594203	594216	+	66	13.61	TTGACCCAGCTCAA	Outer membrane protein OprG	PA4067	*oprG*
PP0625	*clpB*	730328	730341	+	118	13.87	TTGACTTTGGTCAA	Chaperone protein clpB		
PP0762	*hprA*	878339	878352	+	163	13.08	TTGCTGCCCGTCAA	Glycerate dehydrogenase		
PP0763		878339	878352	+	118	13.08	TTGCTGCCCGTCAA	Medium-chain-fatty-acid CoA ligase		
PP0807		947418	947431	+	52	12.92	ATGACCTGCGTCAA	Sigma-54 dependent transcriptional regulator		
PP0808	*hmpA*	947418	947431	+	91	12.92	ATGACCTGCGTCAA	Flavohemoprotein		
PP0858		992886	992899	-	93	13.48	TTGCTCTGGATCAA	Aminotransferase, class I		
PP0865		1003436	1003449	-	43	12.43	ATGTTTCTCATCAA	RNA polymerase sigma-70 factor, putative		
PP0903		1043504	1043517	+	90	11.77	TTGTATCAGGTCAA	Putative uncharacterized protein		
PP0910		1053421	1053434	-	57	12.00	TTGCTCTTATTCAA	Putative uncharacterized protein	PA4371	
PP0914		1058147	1058160	+	78	11.91	ATGCGCTGTATCAA	GGDEF domain protein		
PP0993		1132795	1132808	+	181	11.62	ATGAATCAAGTCAA	Putative uncharacterized protein		
PP0994		1132795	1132808	+	78	11.62	ATGAATCAAGTCAA	RNA polymerase sigma-70 factor, ECF subfamily		
PP1002	*arcD*	1142487	1142500	-	61	11.84	CTGACATAAGTCAG	arginine/ornithine antiporter	PA5170	*arcD*
PP1009	*gap-1*	1151102	1151115	-	93	12.42	TTGTTTTCTATCAG	Glyceraldehyde 3-phosphate dehydrogenase		
PP1010	*edd*	1151102	1151115	-	159	12.42	TTGTTTTCTATCAG	6-phosphogluconate dehydratase		
PP1057		1205835	1205848	-	76	11.55	ATGATAATCATCAG	Transcriptional regulator, PadR family		
PP1089		1248878	1248891	+	143	11.66	TTGCTGAAGATCAG	Putative uncharacterized protein		
PP1090		1248878	1248891	+	211	11.66	TTGCTGAAGATCAG	DNA-binding response regulator, LuxR family		
PP1099		1257150	1257163	-	22	13.02	CTGACCGTTATCAA	Cold-shock domain family protein		
PP1100	*dcd*	1257150	1257163	-	293	13.02	CTGACCGTTATCAA	Deoxycytidine triphosphate deaminase		
PP1115		1274087	1274100	-	4	11.35	TTGCAGAACATCAA	Lipoprotein, putative		
PP1128		1292383	1292396	+	0	12.85	TTGACTATTCTCAA	OmpA family protein		
PP1130		1294461	1294474	+	19	11.49	GTGACAGCCGTCAA	Putative uncharacterized protein		
PP1131		1294461	1294474	+	44	11.49	GTGACAGCCGTCAA	Outer membrane lipoprotein, putative		

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PP1135		1301120	1301133	+	101	12.79	TTGTCAGTAGTCAA	Putative uncharacterized protein		
PP1136		1301120	1301133	+	116	12.79	TTGTCAGTAGTCAA	Putative uncharacterized protein		
PP1143		1308331	1308344	+	135	12.82	TTGAAAGCAATCAA	3-hydroxyisobutyrate dehydrogenase family protein		
PP1149		1316178	1316191	+	68	14.1	TTGACACTGATCAA	Putative uncharacterized protein		
PP1149		1316127	1316140	-	119	13.92	TTGACCTGCGTCAA	Putative uncharacterized protein		
PP1171		1346955	1346968	+	65	11.50	CTGTGTGTTGTCAA	Uronate dehydrogenase		
PP1172		1346955	1346968	+	7	11.50	CTGTGTGTTGTCAA	Putative uncharacterized protein		
PP1199		1374072	1374085	+	121	12.61	TTGAGAGTCATCAG	Putative uncharacterized protein		
PP1200	*kup*	1374072	1374085	+	294	12.61	TTGAGAGTCATCAG	Probable potassium transport system protein kup		
PP1203	*dinP*	1381064	1381077	-	171	12.24	TTGCTTGACATCAG	DNA polymerase IV		
PP1288	*algD*	1474274	1474287	-	56	12.52	CTGTTGTTAATCAA	GDP-mannose 6-dehydrogenase	PA3540	*algD*
PP1306		1494898	1494911	+	30	11.52	TTGCGGTATGTCAG	Pyocin S-type Killer domain protein		
PP1343	*lpxC*	1530198	1530211	+	72	12.24	TTGGTATTCATCAA	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase		
PP1382		1575048	1575061	+	38	12.71	CTGATAGTGATCAG	Membrane protein, putative		
PP1414	*ushA*	1614633	1614646	+	32	12.22	TTGCTGGCGATCAG	5'-nucleotidase		
PP1542		1745012	1745025	-	142	13.35	CTGATCCTCATCAA	Putative uncharacterized protein		
Pp16SF		2548385	2548398	+	289	11.72	CTGTGTTGAGTCAA	16S ribosomal RNA		
PP1703		1899314	1899327	-	72	11.82	ATGAGGTAGATCAG	Assimilatory nitrate reductase/sulfite reductase, putative		
PP1714	*fklB-2*	1914781	1914794	-	23	11.6	ATGAGGGCAATCAG	Peptidyl-prolyl cis-trans isomerase		
PP1744		1945072	1945085	-	162	11.49	GTGAGTCTAATCAA	Putative uncharacterized protein		
PP1745		1945072	1945085	-	32	11.49	GTGAGTCTAATCAA	Oxidoreductase, short chain dehydrogenase/reductase family		
PP1773	*ihfB*	1981238	1981251	+	107	11.64	TTGAATTACTTCAA	Integration host factor subunit beta		
PP1781		1995064	1995077	+	319	13.54	TTGTTTTCCATCAA	O-acyltransferase, putative		
PP1860		2082227	2082240	+	9	12.83	TTGAAAGTCATCAA	Transcriptional regulator, MarR family		
PP1930	*arsR-1*	2177133	2177146	+	178	12.19	TTGGTACCGATCAA	arsenic resistance transcriptional regulator		
PP1940		2195838	2195851	+	177	12.31	CTGCTGGTCATCAA	Methyl-accepting chemotaxis transducer		
PP1950		2206501	2206514	+	53	11.64	TTGTGCTACGTCAG	Putative uncharacterized protein		
PP1951		2207752	2207765	+	21	13.44	TTGCTGTTCATCAA	Oxidoreductase, short chain dehydrogenase / reductase family		

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PP1966	*holB*	2227770	2227783	-	62	12.94	TTGTGCCGCATCAA	DNA polymerase III, delta prime subunit		
PP1984		2250649	2250662	-	114	11.38	CTGTTCCTCATCAG	Transcriptional regulator, LysR family		
PP1985	*leuC*	2250649	2250662	-	5	11.38	CTGTTCCTCATCAG	3-isopropylmalate dehydratase large subunit		
PP2007		2278796	2278809	-	220	12.49	TTGAATTGCCTCAA	P-47-related protein		
PP2008	*fadH*	2278796	2278809	-	49	12.49	TTGAATTGCCTCAA	2,4-dienoyl-CoA reductase FadH		
PP2010		2282640	2282653	+	35	11.67	TTGCTGGCGCTCAG	Cytochrome b561		
PP2062		2345449	2345462	+	105	12.15	CTGCCAGGGATCAA	Putative uncharacterized protein		
PP2073		2359004	2359017	-	162	11.98	TTGTTCGGGTTCAA	Acetyltransferase, GNAT family		
PP2074		2359004	2359017	-	23	11.98	TTGTTCGGGTTCAA	Transcriptional regulator, LysR family		
PP2092	*nasA*	2385196	2385209	+	58	11.74	TTGTCGGGATTCAA	Nitrate transporter		
PP2121		2421097	2421110	-	98	12.97	TTGCCTTGGGTCAA	Lipoprotein, putative		
PP2122	*moaB-1*	2421097	2421110	-	77	12.97	TTGCCTTGGGTCAA	Molybdenum cofactor biosynthesis protein B		
PP2135		2436765	2436778	+	60	11.96	ATGCTTGTGGTCAA	Putative uncharacterized protein		
PP2136	*fadB*	2436765	2436778	+	300	11.96	ATGCTTGTGGTCAA	Fatty acid oxidation complex subunit alpha		
PP2161		2468332	2468345	+	99	13.04	ATGATCCGCGTCAA	Putative uncharacterized protein		
PP2187		2494702	2494715	+	68	14.44	TTGATGTACATCAA	Universal stress protein family	PA1789	*uspL*
PP2188		2494702	2494715	+	74	14.44	TTGATGTACATCAA	tRNA-(Ms(2)io(6)a)-hydroxylase, putative	PA1790	
PP2194		2501476	2501489	-	11	12.14	CTGACATTCATCAG	Transcriptional regulator, LysR family		
PP2195		2501476	2501489	-	70	12.14	CTGACATTCATCAG	Periplasmic polyamine-binding protein, putative		
PP2206		2513194	2513207	+	108	14.12	TTGATTTGCGTCAA	Peptidase, U32 family	PA5440	
PP2207		2513194	2513207	+	66	14.12	TTGATTTGCGTCAA	Membrane protein, putative		
PP2209	*phnW*	2516582	2516595	+	58	12.27	CTGCCATCAATCAA	2-aminoethylphosphonate--pyruvate transaminase		
PP2210		2516582	2516595	+	42	12.27	CTGCCATCAATCAA	Transcriptional regulator, LysR family		
PP2239		2548385	2548398	+	187	11.72	CTGTGTTGAGTCAA	Transporter, EamA family		
PP2259		2578422	2578435	+	31	11.46	CTGTTCATTGTCAA	Sigma-54 dependent transcriptional regulator		
PP2260		2578422	2578435	+	153	11.46	CTGTTCATTGTCAA	Sugar ABC transporter, ATP-binding protein		
PP2282		2603753	2603766	-	101	11.88	CTGTTGCTTCTCAA	Minor capsid protein 10		
PP2295		2623158	2623171	-	236	11.83	ATGACTTGCGTCAG	Antirestriction protein, putative		

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PP2300	*clpP*	2628292	2628305	+	31	11.38	TTGTGCGTATTCAA	ATP-dependent Clp protease proteolytic subunit		
PP2310		2640732	2640745	-	79	14.43	TTGATATGCATCAA	Methyl-accepting chemotaxis transducer	PA2867	
PP2310		2640672	2640685	-	19	12.91	TTGTTTCCGCTCAA	Methyl-accepting chemotaxis transducer	PA2867	
PP2322	*oprI*	2650539	2650552	-	96	11.51	TTGCGCTCTGTCAG	Outer membrane lipoprotein OprI	PA2853	*oprI*
PP2323		2650539	2650552	-	249	11.51	TTGCGCTCTGTCAG	Conserved domain protein		
PP2377		2717636	2717649	-	12	12.08	TTGCAATCGATCAA	Acetyltransferase Act, putative		
Pp23SF		2550534	2550547	+	167	11.79	CTGACTTTTGTCAG	23S ribosomal RNA		
Pp23SG		5310797	5310810	+	210	12.90	CTGAGTTTGATCAA	23S ribosomal RNA		
Pp23SG		5310754	5310767	-	167	11.79	CTGACTTTTGTCAG	23S ribosomal RNA		
PP2420		2769019	2769032	-	303	11.84	TTGTTTTTTCTCAG	Outer membrane ferric siderophore receptor		
PP2444		2792668	2792681	+	133	11.83	ATGCTGCAACTCAA	Transcriptional regulator, LysR family		
PP2445		2792668	2792681	+	255	11.83	ATGCTGCAACTCAA	Putative uncharacterized protein		
PP2449		2796672	2796685	+	164	13.80	TTGATTTTTCTCAA	Putative uncharacterized protein		
PP2452		2799082	2799095	+	99	11.41	ATGAGGCCGGTCAG	Putative uncharacterized protein		
PP2474		2821638	2821651	+	310	12.73	CTGAGTGTCATCAA	Glutathione S-transferase family protein		
PP2475		2821638	2821651	+	68	12.73	CTGAGTGTCATCAA	Transcriptional regulator, TetR family		
PP2557		2905941	2905954	+	136	11.79	TTGCCACCGGTCAG	Sensory box protein		
PP2648		3036476	3036489	-	69	14.12	TTGACCCAAATCAA	Universal stress protein family	PA4352	*uspN*
PP2653		3042038	3042051	-	50	12.72	TTGCTTGCACTCAA	Transcriptional regulator, Cro/CI family		
PP2680		3068773	3068786	-	123	12.10	TTGCCACCGATCAG	Aldehyde dehydrogenase family protein		
PP2688		3079391	3079404	-	129	12.72	CTGATTGTGCTCAA	Putative uncharacterized protein		
PP2745		3128493	3128506	+	80	14.35	TTGATATTTATCAA	Universal stress protein family	PA1789	*uspL*
PP2746		3128493	3128506	+	245	14.35	TTGATATTTATCAA	Putative uncharacterized protein		
PP2840		3245644	3245657	+	153	11.74	TTGACTTGCTTCAG	Membrane protein, putative		
PP2841		3245644	3245657	+	86	11.74	TTGACTTGCTTCAG	Site-specific recombinase, phage integrase family		
PP2874		3275894	3275907	-	77	12.82	ATGAGGCGGATCAA	Putative uncharacterized protein		
PP2875		3276896	3276909	+	204	12.82	ATGAACGGCGTCAA	Putative uncharacterized protein		
PP2900		3297690	3297703	+	101	12.47	CTGTTTCTCATCAA	Putative uncharacterized protein		

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PP2901	*pvdQ*	3297690	3297703	+	78	12.47	CTGTTTCTCATCAA	Acyl-homoserine lactone acylase pvdQ		
PP2908		3307736	3307749	-	141	12.14	ATGACCTGGATCAG	Transcriptional regulator, putative		
PP2908		3307642	3307655	-	47	12.11	TTGTTTTTCGTCAG	Transcriptional regulator, putative		
PP2909		3307736	3307749	-	42	12.14	ATGACCTGGATCAG	hypothetical protein		
PP2909		3307642	3307655	-	136	12.11	TTGTTTTTCGTCAG	hypothetical protein		
PP3010		3402011	3402024	-	81	11.88	ATGTTACTGCTCAA	Putative uncharacterized protein		
PP3080	*aroF-2*	3466068	3466081	-	277	11.53	ATGAGAATACTCAA	phospho-2-dehydro-3-deoxyheptonate aldolase		
PP3082		3468840	3468853	+	140	11.78	TTGCCACCGTTCAA	Membrane protein, putative		
PP3083		3468840	3468853	+	208	11.78	TTGCCACCGTTCAA	Membrane protein, putative		
PP3086		3474128	3474141	+	24	11.44	GTGAGTGCAATCAA	RNA polymerase sigma-70 factor, putative		
PP3101		3498872	3498885	+	113	12.23	CTGCCGCGGATCAA	ADP-ribosylglycohydrolase family protein		
PP3151		3570877	3570890	+	16	11.39	ATGTTGTTAATCAG	Aldehyde dehydrogenase family protein		
PP3152		3570877	3570890	+	79	11.39	ATGTTGTTAATCAG	Transcriptional regulator, LysR family		
PP3164	*benD*	3584857	3584870	-	115	12.36	TTGCTTTGAATCAG	1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase		
PP3178		3602529	3602542	-	50	11.66	GTGACCTCGGTCAA	Putative uncharacterized protein		
PP3184		3614003	3614016	-	323	12.26	ATGATCCGGATCAG	Putative uncharacterized protein		
PP3185	*pet18*	3614003	3614016	-	109	12.26	ATGATCCGGATCAG	transcriptional activator, TenA family		
PP3230		3666079	3666092	+	270	13.47	CTGATCTGCATCAA	Phosphoribosyl transferase domain protein		
PP3232		3669376	3669389	+	64	13.67	TTGACAGTTGTCAA	Acetyltransferase, GNAT family		
PP3232		3669587	3669600	+	275	13.30	TTGATCTTCATCAG	Acetyltransferase, GNAT family	PA5475	
PP3233		3669376	3669389	+	315	13.67	TTGACAGTTGTCAA	Transcriptional regulator, Crp/Fnr family		
PP3233		3669587	3669600	+	104	13.30	TTGATCTTCATCAG	Transcriptional regulator, Crp/Fnr family		
PP3236		3672427	3672440	-	98	14.15	TTGACCTTTATCAA	Lipoprotein OprI, putative		
PP3236		3672465	3672478	-	60	12.87	CTGACATTTGTCAA	Lipoprotein OprI, putative		
PP3237		3672814	3672827	-	123	12.20	TTGGTTTTTATCAA	Universal stress protein family	PA4328	*uspM*
PP3238		3674616	3674629	-	64	12.00	TTGGCTTTTATCAA	Transcriptional regulator PyrR, putative		
PP3239		3674616	3674629	-	155	12.00	TTGGCTTTTATCAA	Tn4652, cointegrate resolution protein T, putative		
PP3288		3722668	3722681	-	60	14.35	TTGATGTTTATCAA	Universal stress protein family	PA4328	*uspM*

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PP3288		3722609	3722622	-	119	14.13	TTGACGCCGATCAA	Universal stress protein family	PA4328	*uspM*
PP3289		3724346	3724359	+	101	13.47	CTGATCTGCATCAA	Acetyltransferase, GNAT family		
PP3289		3724310	3724323	+	65	12.48	CTGACAATCATCAA	Acetyltransferase, GNAT family		
PP3290		3724346	3724359	+	69	13.47	CTGATCTGCATCAA	Universal stress protein family	PA4328	*uspM*
PP3290		3724310	3724323	+	105	12.48	CTGACAATCATCAA	Universal stress protein family	PA4328	*uspM*
PP3293		3728046	3728059	+	132	14.35	TTGATATTTATCAA	Putative uncharacterized protein		
PP3294		3728046	3728059	+	68	14.35	TTGATATTTATCAA	Universal stress protein family	PA4328	*uspM*
PP3297		3729961	3729974	-	198	12.37	CTGATATCGATCAG	Putative uncharacterized protein		
PP3320		3757130	3757143	+	19	11.40	CTGTGCCTGCTCAA	Conserved domain protein		
PP3321		3757130	3757143	+	33	11.40	CTGTGCCTGCTCAA	Putative uncharacterized protein		
PP3379		3824990	3825003	-	32	12.48	CTGCTGTTGATCAA	Xylose isomerase domain protein TIM barrel		
PP3403		3855236	3855249	+	37	13.30	ATGATGCCAATCAA	Putative uncharacterized protein		
PP3497		3965628	3965641	-	164	11.74	TTGCGACCAATCAG	Peptidase, U32 family		
PP3535	*ggt-1*	4007994	4008007	-	122	12.74	TTGCTCGATCTCAA	Gamma-glutamyltransferase		
PP3547		4022169	4022182	+	62	11.40	ATGACGAACATCAG	Oxidoreductase, short chain dehydrogenase/ reductase family		
PP3609		4101625	4101638	-	42	13.29	TTGATTTCTATCAG	Putative uncharacterized protein		
PP3610		4101625	4101638	-	110	13.29	TTGATTTCTATCAG	Putative uncharacterized protein		
PP3630		4127405	4127418	-	49	11.83	CTGCCGGCCGTCAA	Porin, putative		
PP3631		4127405	4127418	-	210	11.83	CTGCCGGCCGTCAA	Putative uncharacterized protein		
PP3658		4155605	4155618	-	116	11.60	TTGGTGCCTCTCAA	Aromatic compound MFS transporter, putative		
PP3686		4192055	4192068	+	111	12.26	CTGTCTCCTATCAA	Putative uncharacterized protein		
PP3706		4228024	4228037	-	185	12.01	CTGCTTGGAGTCAA	Putative uncharacterized protein		
PP3710		4232894	4232907	+	105	12.20	CTGTTACGCGTCAA	Putative uncharacterized protein		
PP3738		4266692	4266705	+	237	12.45	TTGAGGTTCTTCAA	Transcriptional regulator VanR, putative		
PP3741	*mrdA-1*	4269832	4269845	+	14	11.55	TTGCCACCCCTCAG	Penicillin-binding protein 2		
PP3770		4298601	4298614	-	47	12.40	CTGTTTGAAATCAA	Putative uncharacterized protein		
PP3791		4320999	4321012	-	237	12.64	TTGAAATTTGTCAA	Site-specific recombinase, phage integrase family		
PP3915		4423750	4423763	+	3	11.61	ATGAGAGGTATCAG	Putative uncharacterized protein		

PP number	gene name	start	end	strand	ATG distance	PWM Score	sequence of predicted Anr box	function of gene product	*P. aeruginosa* orthologs with predicted Anr box in promoter	
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PP3931		4434782	4434795	+	77	12.01	TTGCAGCACATCAA	Transporter, sodium/sulfate symporter family		
PP3963		4473098	4473111	+	348	12.35	CTGAGATGTCTCAA	Conserved domain protein		
PP4009	*clpS*	4519993	4520006	-	82	12.15	TTGCCGATAGTCAA	ATP-dependent Clp protease adapter protein	PA2621	
PP4010	*cspD*	4519993	4520006	-	136	12.15	TTGCCGATAGTCAA	Cold-shock protein CspD	PA2622	*cspD*
PP4027		4538421	4538434	+	81	11.72	ATGAGGCCCATCAG	Putative uncharacterized protein		
PP4076		4603866	4603879	+	289	11.50	CTGTTCATCGTCAA	Putative uncharacterized protein		
PP4084		4612783	4612796	+	346	11.48	CTGCGCGTCGTCAA	Putative uncharacterized protein		
PP4102		4637198	4637211	-	46	11.97	GTGACCTCGATCAA	Putative uncharacterized protein		
PP4103		4637198	4637211	-	70	11.97	GTGACCTCGATCAA	Low-affinity inorganic phosphate transporter		
PP4106	*gabP*	4641942	4641955	-	144	13.27	CTGACCTGGATCAA	Gamma-aminobutyrate transporter		
PP4107		4641942	4641955	-	99	13.27	CTGACCTGGATCAA	Transcriptional regulator, LysR family		
PP4132		4671565	4671578	-	76	12.14	ATGAGAACCATCAA	Putative uncharacterized protein		
PP4133		4671565	4671578	-	91	12.14	ATGAGAACCATCAA	Transcriptional regulator-related protein		
PP4155		4696347	4696360	+	66	11.56	ATGATCATCATCAG	Putative uncharacterized protein		
PP4156		4696347	4696360	+	12	11.56	ATGATCATCATCAG	Transcriptional regulator, LysR family		
PP4178		4721643	4721656	+	49	13.48	TTGCTGTGCATCAA	Dienelactone hydrolase family protein		
PP4208		4753417	4753430	-	49	11.79	CTGATATCACTCAG	RNA polymerase sigma-70 factor, ECF subfamily		
PP4209		4753417	4753430	-	151	11.79	CTGATATCACTCAG	ABC export system, membrane fusion protein		
PP4259		4842963	4842976	+	176	11.98	GTGACCTGGATCAA	Iron-sulfur cluster-binding protein		
PP4264	*hemN*	4848623	4848636	-	64	14.39	TTGATTTGTATCAA	Oxygen-independent coproporphyrinogen III oxidase	PA1546	*hemN*
PP4271		4856986	4856999	-	183	11.45	TTGTAACTTCTCAA	Putative uncharacterized protein		
PP4299	*glxR*	4889672	4889685	-	21	12.45	TTGTGATATCTCAA	2-hydroxy-3-oxopropionate reductase		
PP4391	*flgB*	4983716	4983729	-	168	13.18	TTGCCGCCCATCAA	Flagellar basal-body rod protein FlgB		
PP4405		4998110	4998123	-	12	12.23	TTGCTGGGCATCAG	Sensory box protein		
PP4406		4998110	4998123	-	191	12.23	TTGCTGGGCATCAG	Putative uncharacterized protein		
PP4425		5021635	5021648	+	38	12.82	CTGACACCTGTCAA	Amino acid ABC transporter, ATP-binding protein		
PP4433		5029099	5029112	+	248	11.91	CTGAAACTTATCAA	Amino acid MFS transporter		
PP4434	*dadA-1*	5030892	5030905	+	302	13.21	TTGCCCTTAATCAA	D-amino acid dehydrogenase 1 small subunit	PA5304	*dadA*

PP number	gene name	start	end	strand	ATG distance	PWM Score	sequence of predicted Anr box	function of gene product	*P. aeruginosa* orthologs with predicted Anr box in promoter	
									PA number	gene name
PP4469	*gmk-1*	5076728	5076741	-	190	13.00	TTGCTTGGCGTCAA	Guanylate kinase		
PP4470	*algZ*	5076728	5076741	-	136	13.00	TTGCTTGGCGTCAA	alginate biosynthesis transcriptional activator	PA3385	*amrZ*
PP4471	*mgtE*	5079023	5079036	-	270	12.23	CTGAGCGGCCTCAA	Magnesium transporter		
PP4489	*phhR*	5101641	5101654	+	17	11.98	CTGATGTTTTTCAA	Sigma-54 dependent transcriptional regulator PhhR		
PP4490	*phhA*	5101641	5101654	+	228	11.98	CTGATGTTTTTCAA	Phenylalanine-4-hydroxylase		
PP4521		5136617	5136630	-	50	12.40	CTGCTACATATCAA	Aerotaxis receptor, putative		
PP4538		5155400	5155413	-	335	12.40	CTGACCGGGGTCAA	FMN-dependent NADH-azoreductase 2		
PP4539		5155400	5155413	-	127	12.79	CTGACCGGGGTCAA	Transcriptional regulator, LysR family		
PP4570		5190411	5190424	+	148	11.78	TTGCGCCGGATCAG	Putative uncharacterized protein		
PP4651	*cioA*	5276840	5276853	+	146	14.15	TTGACCCAGATCAA	Ubiquinol oxidase subunit I, cyanide insensitive	PA3930	*cioA*
PP4652		5276840	5276853	+	185	14.15	TTGACCCAGATCAA	Membrane protein, putative		
PP4714		5361024	5361037	+	188	11.53	CTGTTGAGGGTCAA	hypothetical, unclassified, unknown		
PP4734		5383505	5383518	-	227	12.11	TTGTTGTTGGTCAG	Transcriptional regulator, GntR family		
PP4735	*lctP*	5383505	5383518	-	17	12.11	TTGTTGTTGGTCAG	L-lactate transporter		
PP4739		5390598	5390611	-	156	12.51	TTGCGGCCTGTCAA	Putative uncharacterized protein		
PP4870		5537635	5537648	+	63	13.16	CTGATATGGGTCAA	Azurin	PA4922	*azu*
PP4871		5537635	5537648	+	272	13.16	CTGATATGGGTCAA	Putative uncharacterized protein	PA4923	
PP4922	*thiC*	5596264	5596277	-	191	11.83	TTGAACCTGATCAG	Thiamine biosynthesis protein thiC		
PP4923		5596264	5596277	-	218	11.83	TTGAACCTGATCAG	TolC family type I secretion outer membrane protein		
PP4933		5613020	5613033	-	12	11.47	TTGCGTCGGGTCAG	Putative uncharacterized protein		
PP5061		5773111	5773124	+	158	11.65	ATGCCACGCCTCAA	Choline/carnitine/betaine transporter family protein		
PP5074	*hemE*	5791793	5791806	+	85	12.20	ATGCCACAGATCAA	Uroporphyrinogen decarboxylase		
PP5206		5936402	5936415	-	60	14.11	TTGATGTCGGTCAA	Membrane fusion protein	PA5232	
PP5211		5944256	5944269	+	31	13.08	CTGACCGAAATCAA	ChaC-related protein		
PP5215	*trx-2*	5949173	5949186	+	152	13.28	TTGCCTTGCATCAA	Thioredoxin		
PP5216	*ppx*	5949173	5949186	+	89	13.28	TTGCCTTGCATCAA	Exopolyphosphatase		
PP5258		6003963	6003976	+	36	12.34	ATGATGTGGATCAG	Aldehyde dehydrogenase family protein		
PP5259		6003963	6003976	+	87	12.34	ATGATGTGGATCAG	Transcriptional regulator, LysR family		

PP number	gene name	start	end	strand	ATG distance	PWM Score	sequence of predicted Anr box	function of gene product	*P. aeruginosa* orthologs with predicted Anr box in promoter	
									PA number	gene name
PP5337		6084860	6084873	+	150	11.63	ATGTGCCGGGTCAA	Transcriptional regulator, LysR family		
PP5338	*aspA*	6084860	6084873	+	8	11.63	ATGTGCCGGGTCAA	aspartate ammonia-lyase		
PP5374		6125169	6125182	-	14	12.10	TTGTTTTTGTTCAA	Choline/carnitine/betaine transporter family protein		
PP5376		6128682	6128695	+	101	11.67	TTGCCAGGTGTCAG	Putative uncharacterized protein		
PP5395		6150832	6150845	+	135	11.82	GTGACGCTAATCAA	Putative uncharacterized protein		

Appendix G

***Pseudomonas putida* KT2440 genes differentially regulated in wild type, but not Δ*anr* mutant strain, after 30 minutes of oxygen depletion of mid-exponential phase planktonic cultures.** Experimental procedures and data analysis are described elsewhere (3.2.2.2). PP number, gene name, description of encoded proteins and functional classification are according to "*Pseudomonas* Genome Database". Enlisted are fold change expression values for wild type after 30 minutes of oxygen depletion compared to wild type prior to anaerobic shift ("FC wt anaerobic") and for Δ*anr* mutant strain after 30 minutes of oxygen depletion compared to wild type prior to anaerobic shift ("FC Δanr anaerobic"). Fold change expression values lower than 1.75-fold and higher than 0.57-fold are not enlisted ("/"). Genes marked with a plus sign (+) were identified as Anr-dependent by "Virtual Footprint – Regulon Analysis" (Appendix F), genes marked with a rhombus (#) were differentially regulated by Anr in *P. aeruginosa* in response to oxygen depletion in a similar experiment (Trunk & Benkert *et al*., 2010).

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
52.35	0.35	PP5391		hypothetical protein	Hypothetical, unclassified, unknown
37.27	/	PP2187 # +		universal stress protein family	Adaptation & protection
32.00	0.35	PP1002 # +	*arcD*	arginine/ornithine antiporter	Transport of small molecules
31.56	/	PP1001 #	*arcA*	arginine deiminase	Energy metabolism
29.65	/	PP1000 #	*argI*	ornithine carbamoyltransferase, catabolic	Energy metabolism
24.42	2.10	PP2161 +		conserved hypothetical protein	Hypothetical, unclassified, unknown
22.78	/	PP0273 # +		conserved hypothetical protein	Hypothetical, unclassified, unknown
22.63	0.40	PP3839 #	*adhA*	alcohol dehydrogenase, zinc-containing	Energy metabolism
22.32	/	PP2121 +		lipoprotein, putative	Cell wall, LPS & capsule
21.71	/	PP0998 #		conserved hypothetical protein	Hypothetical, unclassified, unknown
20.97	0.42	PP2648 # +		universal stress protein family	Adaptation & protection
20.25	0.42	PP4253	*ccoP-1*	cytochrome c oxidase, cbb3-type, subunit III	Energy metabolism
20.11	0.53	PP3823		cytochrome c-type protein	Energy metabolism
18.00	0.21	PP4250	*ccoN-1*	cytochrome c oxidase, cbb3-type, subunit I	Energy metabolism
18.00	/	PP5389		hypothetical protein	Hypothetical, unclassified, unknown
17.03	0.08	PP0504 # +	*oprG*	outer membrane protein OprG	Cell wall, LPS & capsule

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
16.80	0.43	PP4870 +		azurin	Biosynthesis of cofactors, prosthetic groups & carriers
15.67	/	PP0999 #	*arcC*	carbamate kinase	Energy metabolism
15.35	/	PP5390		hypothetical protein	Hypothetical, unclassified, unknown
14.83	2.25	PP0181		conserved hypothetical protein	Hypothetical, unclassified, unknown
13.74	0.24	PP4251 #	*ccoO-1*	cytochrome c oxidase, cbb3-type, subunit II	Energy metabolism
13.55	2.60	PP0625 +	*clpB*	ATP-dependent Clp protease, ATP-binding subunit ClpB	Translation, post-translational modification & degradation
13.27	1.87	PP1211		conserved hypothetical protein	Hypothetical, unclassified, unknown
12.73	/	PP3822		cytochrome c family protein	Energy metabolism
11.55	/	PP4260		conserved hypothetical protein	Hypothetical, unclassified, unknown
10.63	2.13	PP1210		DNA-binding stress protein, putative	Adaptation & protection
10.41	/	PP4261		cation-transporting P-type ATPase	Transport of small molecules
10.13	0.27	PP2874 +		hypothetical protein	Hypothetical, unclassified, unknown
9.51	4.44	PP4434 +	*dadA-1*	D-amino acid dehydrogenase, small subunit	Amino acid biosynthesis & metabolism
9.45	2.08	PP5358		conserved hypothetical protein	Hypothetical, unclassified, unknown
9.38	0.44	PP5207 #		ABC transporter, ATP-binding protein/permease protein, putative	Transport of small molecules
9.32	/	PP1318	*petB*	ubiquinol--cytochrome c reductase, cytochrome b	Energy metabolism
9.13	/	PP3288 # +		universal stress protein family	Adaptation & protection
8.94	/	PP3234		heat shock protein, HSP20 family	Chaperones & heat shock proteins
8.94	/	PP3236 +		lipoprotein OprI, putative	Cell wall, LPS & capsule
8.88	/	PP1130 +		hypothetical protein	Hypothetical, unclassified, unknown
8.28	/	PP0490		formate dehydrogenase, iron-sulfur subunit	Energy metabolism
7.84	/	PP3840		hypothetical protein	Hypothetical, unclassified, unknown
7.78	3.01	PP2213	*fadDx*	acyl-CoA ligase	Fatty acid & phospholipid metabolism
7.67	0.37	PP4252	*ccoQ-1*	cytochrome c oxidase, cbb3-type, CcoQ subunit	Energy metabolism
7.46	/	PP0585		transcriptional regulator, MerR family	Transcriptional regulators
7.36	0.19	PP5206 +		membrane fusion protein	Membrane proteins
7.21	/	PP0492		formate dehydrogenase accessory protein FdhE, putative	Energy metabolism
7.21	/	PP5393		heavy metal transport/detoxification protein	Transport of small molecules
7.06	/	PP0052		metallo-beta-lactamase family protein	Putative enzymes

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
6.92	/	PP3290 # +		universal stress protein family	Adaptation & protection
6.87	3.12	PP1821		glutathione S-transferase family protein	Putative enzymes
6.50	/	PP4259 +		iron-sulfur cluster-binding protein	Energy metabolism
6.50	0.33	PP4264 # +	*hemN*	oxygen-independent coproporphyrinogen III oxidase	Biosynthesis of cofactors, prosthetic groups & carriers
6.45	2.87	PP0191	*pfrA*	anti-RNA polymerase sigma 70 factor	Transcriptional regulators
6.36	/	PP1153		lipoprotein, putative	Cell wall, LPS & capsule
6.23	2.60	PP0126		cytochrome c4	Energy metabolism
6.11	/	PP0545		aldehyde dehydrogenase family protein	Putative enzymes
6.06	/	PP5267		cytochrome c5	Energy metabolism
6.02	/	PP3233.1		unknown	Hypothetical, unclassified, unknown
5.86	/	PP5208		ABC transporter, permease protein	Transport of small molecules
5.74	/	PP1522	*cspA-1*	cold shock protein CspA	Adaptation & protection
5.70	/	PP4642		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.66	/	PP5394		unknown	Hypothetical, unclassified, unknown
5.46	2.53	PP2080		NAD-glutamate dehydrogenase	Putative enzymes
5.39	/	PP3235		conserved domain protein	Hypothetical, unclassified, unknown
5.39	/	PP3640		transcriptional regulator, AraC family	Transcriptional regulators
5.21	2.48	PP4847		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.17	/	PP0586		heavy metal translocating P-type ATPase	Transport of small molecules
5.13	/	PP3720		NAD(P)H quinone oxidoreductase, putative	Energy metabolism
5.13	/	PP4103 +		low-affinity inorganic phosphate transporter	Transport of small molecules
5.10	/	PP0489		unknown	Hypothetical, unclassified, unknown
5.06	/	PP0122		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.03	/	PP0430		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.89	/	PP0431 +		conserved hypothetical protein TIGR00701	Hypothetical, unclassified, unknown
4.82	/	PP3232 # +		acetyltransferase, GNAT family	Putative enzymes
4.82	/	PP3610 +		hypothetical protein	Hypothetical, unclassified, unknown
4.82	/	PP4322	*ccmF*	cytochrome c-type biogenesis protein CcmF	Energy metabolism
4.66	/	PP3231		conserved domain protein	Hypothetical, unclassified, unknown

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
4.59	2.11	PP3380	*ptxS*	transcriptional regulator PtxS	Transcriptional regulators
4.53	/	PP0563		response regulator receiver modulated diguanylate cyclase	Two-component regulatory systems
4.32	2.08	PP2921		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.32	1.78	PP4115		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.29	/	PP0427		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.26	/	PP0608		hypothetical protein	Hypothetical, unclassified, unknown
4.26	2.11	PP4479	*aruG*	arginine N-succinyltransferase, beta subunit	Amino acid biosynthesis & metabolism
4.20	/	PP3696		hypothetical protein	Hypothetical, unclassified, unknown
4.14	/	PP3580		hypothetical protein	Hypothetical, unclassified, unknown
4.08	/	PP0491		formate dehydrogenase, cytochrome b556 subunit	Energy metabolism
4.08	/	PP2459		ribose ABC transporter, periplasmic ribose-binding protein	Transport of small molecules
4.06	/	PP0053		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.03	1.83	PP0202 +		CBS domain protein	Hypothetical, unclassified, unknown
3.97	/	PP1729		hypothetical protein	Hypothetical, unclassified, unknown
3.94	/	PP3547 +		short chain dehydrogenase/reductase oxidoreductase	Putative enzymes
3.94	/	PP3721	*aspC*	aspartate aminotransferase	Amino acid biosynthesis & metabolism
3.94	/	PP4325	*ccmC*	heme ABC export system, permease protein CcmC	Transport of small molecules
3.92	/	PP4321	*dsbE*	thiol:disulfide interchange protein DsbE	Energy metabolism
3.86	/	PP3722	*alr*	alanine racemase, putative	Amino acid biosynthesis & metabolism
3.86	/	PP4323	*ccmE*	cytochrome c-type biogenesis protein CcmE	Energy metabolism
3.84	/	PP1003 #		arginine/ornithine antiporter	Transport of small molecules
3.78	/	PP3237 # +		universal stress protein family	Adaptation & protection
3.78	/	PP4320		unknown	Hypothetical, unclassified, unknown
3.76	/	PP1317	*petA*	ubiquinol--cytochrome c reductase, iron-sulfur subunit	Energy metabolism
3.66	/	PP0859		carbon-nitrogen hydrolase family protein	Putative enzymes
3.63	/	PP0185	*pprA*	LytTR family two component transcriptional regulator	Two-component regulatory systems
3.63	/	PP3292		hypothetical protein	Hypothetical, unclassified, unknown
3.61	/	PP2680 +		aldehyde dehydrogenase family protein	Putative enzymes
3.58	/	PP4319		hypothetical protein	Hypothetical, unclassified, unknown

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
3.53	/	PP0125		cytochrome c-type protein	Energy metabolism
0.29	/	PP4849		DnaK-protein, putative	Chaperones & heat shock proteins
0.28	/	PP1251	*mqo-2*	malate:quinone oxidoreductase	Energy metabolism
0.28	/	PP4944		carbamoyltransferase, NodU family	Putative enzymes
0.28	/	PP1179	*nrdA*	ribonucleoside reductase, alpha subunit	Nucleotide biosynthesis & metabolism
0.28	/	PP0963	*ttg2F*	toluene-tolerance protein	Adaptation & protection
0.28	/	PP5338 +	*aspA*	aspartate ammonia-lyase	Amino acid biosynthesis & metabolism
0.28	/	PP0685		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.28	/	PP4963	*pgk*	phosphoglycerate kinase	Carbon compound catabolism
0.28	/	PP0748		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.27	0.56	PP0960	*ttg2C*	toluene tolerance ABC transporter, periplasmic substrate-binding protein	Adaptation & protection
0.27	0.54	PP4495		aromatic amino acid transporter	Transport of small molecules
0.27	/	PP1788		hypothetical protein	Hypothetical, unclassified, unknown
0.27	0.54	PP0567	*speA*	biosynthetic arginine decarboxylase	Amino acid biosynthesis & metabolism
0.27	/	PP1794		hypothetical protein	Hypothetical, unclassified, unknown
0.26	/	PP0964	*murA*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Cell wall, LPS & capsule
0.26	0.53	PP5213		conserved hypothetical protein TIGR00148	Hypothetical, unclassified, unknown
0.26	/	PP4723	*carB*	carbamoyl-phosphate synthase, large subunit	Nucleotide biosynthesis & metabolism
0.26	/	PP0814	*cyoC*	cytochrome o ubiquinol oxidase, subunit III	Energy metabolism
0.26	0.53	PP4976		unknown	Hypothetical, unclassified, unknown
0.26	0.52	PP0244		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.26	0.54	PP1037	*purL*	phosphoribosylformylglycinamidine synthase	Nucleotide biosynthesis & metabolism
0.26	0.53	PP1792		glycosyl transferase, group 2 family protein	Putative enzymes
0.26	0.52	PP0436	*tyrS*	tyrosyl-tRNA synthetase	Translation, post-translational modification & degradation
0.25	0.50	PP1627		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.25	0.50	PP1367	*purU*	formyltetrahydrofolate deformylase	Nucleotide biosynthesis & metabolism
0.25	/	PP1461	*ffh*	signal recognition particle protein Ffh	Protein secretion & export apparatus
0.25	/	PP0702		major facilitator family transporter	Transport of small molecules
0.25	/	PP4802		conserved hypothetical protein	Hypothetical, unclassified, unknown

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
0.24	/	PP1304	*cysNC*	sulfate adenylyltransferase, subunit 1/adenylylsulfate kinase	Amino acid biosynthesis & metabolism
0.24	0.53	PP1222	*tolB*	tolB protein	Transport of small molecules
0.24	/	PP1609		hypothetical protein	Hypothetical, unclassified, unknown
0.24	0.49	PP0842	*iscS-1*	cysteine desulfurase	Amino acid biosynthesis & metabolism
0.24	/	PP0815	*cyoD*	cytochrome o ubiquinol oxidase, protein CyoD	Energy metabolism
0.23	/	PP1088	*argG*	argininosuccinate synthase	Amino acid biosynthesis & metabolism
0.23	0.53	PP0558	*accC-1*	acetyl-CoA carboxylase, biotin carboxylase	Fatty acid & phospholipid metabolism
0.23	/	PP4820		TIM-barrel protein, putative, NifR3 family	Hypothetical, unclassified, unknown
0.23	0.48	PP4821	*fis*	DNA-binding protein Fis	Transcriptional regulators
0.23	0.47	PP1031	*guaB*	inosine-5-monophosphate dehydrogenase	Nucleotide biosynthesis & metabolism
0.23	/	PP1791		aldolase/synthase, putative	Putative enzymes
0.22	0.56	PP1601	*lpxD*	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	Cell wall, LPS & capsule
0.22	0.50	PP1996	*accD*	acetyl-CoA carboxylase, carboxyl transferase, beta subunit	Fatty acid & phospholipid metabolism
0.22	/	PP0813	*cyoB*	cytochrome o ubiquinol oxidase, subunit I	Energy metabolism
0.22	0.48	PP0674		ABC transporter, ATP-binding protein	Transport of small molecules
0.22	0.46	PP4965	*tktA*	transketolase	Energy metabolism
0.21	0.44	PP1605	*rnhB*	ribonuclease HII	DNA replication, recombination, modification & repair
0.21	0.44	PP0841		BadM/Rrf2 family transcriptional regulator	Transcriptional regulators
0.21	0.51	PP1594	*frr*	ribosome recycling factor	Translation, post-translational modification & degradation
0.21	0.44	PP0560	*aroQ-1*	3-dehydroquinate dehydratase, type II	Amino acid biosynthesis & metabolism
0.21	/	PP0816	*cyoE-2*	protoheme IX farnesyltransferase	Energy metabolism
0.21	/	PP4962		hypothetical protein	Hypothetical, unclassified, unknown
0.21	0.42	PP0933	*mreB*	rod shape-determining protein MreB	Cell division
0.21	/	PP1595	*uppS*	undecaprenyl diphosphate synthase	Cell wall, LPS & capsule
0.20	0.41	PP0519	*thiL*	thiamine monophosphate kinase	Biosynthesis of cofactors, prosthetic groups & carriers
0.20	/	PP1822		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.20	0.46	PP1224		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.20	/	PP1790		acylneuraminate cytidylyltransferase, putative	Putative enzymes
0.20	/	PP0838	*suhB*	extragenic suppressor protein SuhB	Translation, post-translational modification & degradation

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
0.19	/	PP1157		acetolactate synthase, catabolic, putative	Putative enzymes
0.19	/	PP1612	*eno*	enolase	Carbon compound catabolism
0.19	/	PP3365		acetolactate synthase, catabolic, putative	Putative enzymes
0.18	0.42	PP1916	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase II	Fatty acid & phospholipid metabolism
0.18	/	PP0812	*cyoA*	cytochrome o ubiquinol oxidase, subunit II	Energy metabolism
0.18	0.41	PP0440	*tuf-1*	translation elongation factor Tu	Translation, post-translational modification & degradation
0.18	/	PP0811	*cyoups2*	cyoups2 protein	Hypothetical, unclassified, unknown
0.15	0.42	PP1214		conserved hypothetical protein TIGR01033	Hypothetical, unclassified, unknown
0.15	0.33	PP5335	*purK*	phosphoribosylaminoimidazole carboxylase, ATPase subunit	Nucleotide biosynthesis & metabolism
0.15	0.46	PP5336	*purE*	phosphoribosylaminoimidazole carboxylase, catalytic subunit	Nucleotide biosynthesis & metabolism
0.15	0.51	PP0559	*accB*	acetyl-CoA carboxylase, biotin carboxyl carrier protein	Fatty acid & phospholipid metabolism
0.15	0.34	PP0448	*rpoC*	DNA-directed RNA polymerase, beta' subunit	Transcription, RNA processing & degradation
0.14	0.35	PP0441	*secE*	preprotein translocase, SecE subunit	Protein secretion & export apparatus
0.13	0.36	PP4714 +		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.13	0.42	PP1789		hydrolase, haloacid dehalogenase-like family	Putative enzymes
0.13	0.26	PP0872	*prfC*	peptide chain release factor 3	Translation, post-translational modification & degradation
0.12	0.30	PP4715	*tpiA*	triosephosphate isomerase	Central intermediary metabolism
0.11	0.32	PP0443	*rplK*	ribosomal protein L11	Translation, post-translational modification & degradation
0.11	0.28	PP0008	*rnpA*	ribonuclease P protein component	Translation, post-translational modification & degradation
0.11	0.32	PP4822	*purH*	phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	Nucleotide biosynthesis & metabolism
0.11	0.24	PP0480	*rplQ*	ribosomal protein L17	Translation, post-translational modification & degradation
0.11	0.24	PP0690		GTP-binding protein, GTP1/Obg family	Adaptation & Protection
0.11	0.31	PP0722	*prsA*	ribose-phosphate pyrophosphokinase	Nucleotide biosynthesis & metabolism
0.11	0.40	PP0442	*nusG*	transcription antitermination protein NusG	Transcription, RNA processing & degradation
0.10	0.20	PP0719		GTP-dependent nucleic acid-binding protein EngD	Translation, post-translational modification & degradation
0.10	0.21	PP5417	*atpF*	ATP synthase F0, B subunit	Energy metabolism
0.10	0.22	PP5414	*atpG*	ATP synthase F1, gamma subunit	Energy metabolism
0.09	0.33	PP4713	*nusA*	N utilization substance protein A	Transcription, RNA processing & degradation

FC wt anaerobic	FC Δanr anaerobic	PP number	gene name	function of gene product	functional classification
0.07	0.21	PP0447	*rpoB*	DNA-directed RNA polymerase, beta subunit	Transcription, RNA processing & degradation
0.07	0.15	PP4877	*rpsF*	ribosomal protein S6	Translation, post-translational modification & degradation
0.07	0.14	PP0476	*rpsM*	ribosomal protein S13	Translation, post-translational modification & degradation
0.06	0.13	PP0688	*rplU*	ribosomal protein L21	Translation, post-translational modification & degradation
0.06	0.16	PP0600	*rpsT*	ribosomal protein S20	Translation, post-translational modification & degradation
0.06	0.13	PP1772	*rpsA*	ribosomal protein S1	Translation, post-translational modification & degradation
0.06	0.12	PP1591	*rpsB*	ribosomal protein S2	Translation, post-translational modification & degradation
0.06	0.12	PP0470	*rplR*	ribosomal protein L18	Translation, post-translational modification & degradation

Appendix H

***Pseudomonas putida* KT2440 genes differentially regulated in wild type, but not Δ*relA*Δ*spoT* mutant strain, after 30 minutes of oxygen depletion of mid-exponential phase planktonic cultures.** Experimental procedures and data analysis are described elsewhere (3.2.2.2). PP number, gene name, description of encoded proteins and functional classification are according to the "*Pseudomonas* Genome Database". Enlisted are fold change expression values for wild type after 30 minutes of oxygen depletion compared to wild type prior to anaerobic shift ("FC wt anaerobic") and for Δ*relA*Δ*spoT* mutant strain after 30 minutes of oxygen depletion compared to wild type prior to anaerobic shift ("FC Δr/Δs anaerobic"). Fold change expression values lower than 1.75-fold and higher than 0.57-fold are not enlisted ("/").

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
52.35	11.00	PP5391		hypothetical protein	Hypothetical, unclassified, unknown
37.53	18.13	PP2095.1		ribosome modulation factor, putative	Translation, post-translational modification & degradation
37.27	12.91	PP2187		universal stress protein family	Adaptation & protection
32.00	11.31	PP1002	*arcD*	arginine/ornithine antiporter	Transport of small molecules
24.42	7.41	PP2161		conserved hypothetical protein	Hypothetical, unclassified, unknown
22.78	6.45	PP0273		conserved hypothetical protein	Hypothetical, unclassified, unknown
22.32	9.19	PP2121		lipoprotein, putative	Cell wall, LPS & capsule
20.25	8.46	PP4253	*ccoP-1*	cytochrome c oxidase, cbb3-type, subunit III	Energy metabolism
20.11	5.82	PP3823		cytochrome c-type protein	Energy metabolism
18.00	6.82	PP4250	*ccoN-1*	cytochrome c oxidase, cbb3-type, subunit I	Energy metabolism
18.00	7.26	PP5389		hypothetical protein	Hypothetical, unclassified, unknown
17.03	4.56	PP0504	*oprG*	outer membrane protein OprG	Cell wall, LPS & capsule
16.80	6.41	PP4870		azurin	Biosynthesis of cofactors, prosthetic groups & carriers
15.35	5.74	PP5390		hypothetical protein	Hypothetical, unclassified, unknown
13.74	6.92	PP4251	*ccoO-1*	cytochrome c oxidase, cbb3-type, subunit II	Energy metabolism
12.38	2.53	PP1291		PhoH family protein	Hypothetical, unclassified, unknown
11.55	4.56	PP4260		conserved hypothetical protein	Hypothetical, unclassified, unknown
10.48	3.58	PP0383		tryptophan 2-monooxygenase, putative	Amino acid biosynthesis & metabolism
10.41	4.44	PP4261		cation-transporting P-type ATPase	Transport of small molecules

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
10.13	4.69	PP2874		hypothetical protein	Hypothetical, unclassified, unknown
9.51	4.23	PP4434	*dadA-1*	D-amino acid dehydrogenase, small subunit	Amino acid biosynthesis & metabolism
9.45	3.58	PP5358		conserved hypothetical protein	Hypothetical, unclassified, unknown
9.38	4.38	PP0951	*rpoX*	sigma54 modulation protein	Translation, post-translational modification & degradation
9.38	3.89	PP5207		ABC transporter, ATP-binding protein/permease protein, putative	Transport of small molecules
9.32	2.69	PP2132		universal stress protein family	Adaptation & protection
9.32	4.17	PP1318	*petB*	ubiquinol--cytochrome c reductase, cytochrome b	Energy metabolism
9.25	3.23	PP3765	*turB*	transcriptional regulator MvaT family	Transcriptional regulators
9.13	4.26	PP3288		universal stress protein family	Adaptation & protection
9.00	2.93	PP0397		conserved hypothetical protein	Hypothetical, unclassified, unknown
8.82	3.66	PP1487		conserved hypothetical protein	Hypothetical, unclassified, unknown
8.75	3.86	PP0679		conserved hypothetical protein	Hypothetical, unclassified, unknown
8.34	2.48	PP5008		polyhydroxyalkanoate granule-associated protein GA1	Central intermediary metabolism
7.94	2.23	PP5007		polyhydroxyalkanoate granule-associated protein GA2	Central intermediary metabolism
7.78	3.10	PP2213	*fadDx*	acyl-CoA ligase	Fatty acid & phospholipid metabolism
7.78	2.43	PP1149		hypothetical protein	Hypothetical, unclassified, unknown
7.36	2.75	PP5206		membrane fusion protein	Membrane proteins
7.21	2.66	PP5393		conserved hypothetical protein	Hypothetical, unclassified, unknown
7.06	/	PP0052		metallo-beta-lactamase family protein	Putative enzymes
6.50	2.35	PP4264	*hemN*	oxygen-independent coproporphyrinogen III oxidase	Biosynthesis of cofactors, prosthetic groups & carriers
6.45	2.91	PP0191	*pfrA*	anti-RNA polymerase sigma 70 factor	Transcriptional regulators
6.23	/	PP4626		LrgA family protein	Hypothetical, unclassified, unknown
6.06	2.31	PP5267		cytochrome c5	Energy metabolism
5.94	2.95	PP5118		thiosulfate sulfurtransferase, putative	Putative enzymes
5.58	/	PP5279		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.17	2.36	PP2922		urea amidolyase-related protein	Central intermediary metabolism
5.17	1.88	PP4593		conserved hypothetical protein	Hypothetical, unclassified, unknown
5.13	2.73	PP4103		low-affinity inorganic phosphate transporter	Transport of small molecules
5.10	2.43	PP0541		acetyltransferase, GNAT family	Putative enzymes

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
4.99	2.41	PP5347	*accC-2*	acetyl-CoA carboxylase, biotin carboxylase	Fatty acid & phospholipid metabolism
4.82	2.36	PP0382		Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase	Amino acid biosynthesis & metabolism
4.76	/	PP3662		decarboxylase family protein	Putative enzymes
4.59	/	PP3380	*ptxS*	transcriptional regulator PtxS	Transcriptional regulators
4.50	/	PP4036		unknown	Hypothetical, unclassified, unknown
4.32	/	PP2168	*tal*	transaldolase	Carbon compound catabolism
4.32	/	PP2921		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.32	/	PP5211		ChaC-related protein	Hypothetical, unclassified, unknown
4.29	/	PP2920		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.26	/	PP4479	*aruG*	arginine N-succinyltransferase, beta subunit	Amino acid biosynthesis & metabolism
4.26	/	PP1774		hypothetical protein	Hypothetical, unclassified, unknown
4.20	/	PP0742		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.20	/	PP0227		cysteine ABC transporter, periplasmic cysteine-binding protein, putative	Transport of small molecules
4.17	/	PP0201.1		unknown	Hypothetical, unclassified, unknown
4.11	/	PP5182		aminotransferase, class III	Putative enzymes
4.06	/	PP0053		conserved hypothetical protein	Hypothetical, unclassified, unknown
4.03	/	PP0202		CBS domain protein	Hypothetical, unclassified, unknown
4.00	/	PP0201		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.97	/	PP0395		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.89	/	PP4234		unknown	Hypothetical, unclassified, unknown
3.89	/	PP3694		hypothetical protein	Hypothetical, unclassified, unknown
3.86	/	PP1308	*mdeA*	methionine gamma-lyase	Amino acid biosynthesis & metabolism
3.84	/	PP0799.1		unknown	Hypothetical, unclassified, unknown
3.84	/	PP0925		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.84	/	PP1003		arginine/ornithine antiporter	Transport of small molecules
3.81	/	PP0158	*gcdH*	glutaryl-CoA dehydrogenase	Fatty acid & phospholipid metabolism
3.76	/	PP3533		ornithine cyclodeaminase	Amino acid biosynthesis & metabolism
3.73	11.79	PP1982	*ibpA*	heat-shock protein IbpA	Chaperones & heat shock proteins
3.73	/	PP0396		conserved hypothetical protein	Hypothetical, unclassified, unknown

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
3.71	/	PP4010	*cspD*	cold-shock protein CspD	Adaptation & protection
3.71	/	PP0788		conserved hypothetical protein	Hypothetical, unclassified, unknown
3.68	/	PP0680		ATP-dependent protease, putative	Translation, post-translational modification & degradation
3.66	/	PP0228		serine O-acetyltransferase, putative	Amino acid biosynthesis & metabolism
3.63	/	PP4233		oxidoreductase, small subunit, putative	Putative enzymes
3.63	/	PP4975		long-chain acyl-CoA thioester hydrolase family protein	Putative enzymes
3.63	/	PP0185	*pprA*	LytTR family two component transcriptional regulator	Two-component regulatory systems
3.51	/	PP0738		conserved domain protein	Hypothetical, unclassified, unknown
0.29	/	PP4849		DnaK-protein, putative	Chaperones & heat shock proteins
0.29	/	PP0059		D,D-heptose 1,7-bisphosphate phosphatase	Cell wall, LPS & capsule
0.28	/	PP4944		carbamoyltransferase, NodU family	Putative enzymes
0.28	/	PP1604	*lpxB*	lipid A disaccharide synthase	Cell wall, LPS & capsule
0.28	/	PP1179	*nrdA*	ribonucleoside reductase, alpha subunit	Nucleotide biosynthesis & metabolism
0.28	/	PP0685		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.28	/	PP0687	*ispB*	octylprenyl diphosphate synthase	Biosynthesis of cofactors, prosthetic groups & carriers
0.28	/	PP4963	*pgk*	phosphoglycerate kinase	Carbon compound catabolism
0.27	/	PP3992		xanthine/uracil permease family protein	Transport of small molecules
0.27	/	PP1794		hypothetical protein	Hypothetical, unclassified, unknown
0.26	/	PP4723	*carB*	carbamoyl-phosphate synthase, large subunit	Nucleotide biosynthesis & metabolism
0.26	/	PP0814	*cyoC*	cytochrome o ubiquinol oxidase, subunit III	Energy metabolism
0.26	/	PP0857	*engA*	GTP-binding protein EngA	Translation, post-translational modification & degradation
0.26	/	PP0403	*surA*	survival protein SurA	Adaptation & protection
0.26	/	PP4803	*dacA*	D-alanyl-D-alanine carboxypeptidase	Cell wall, LPS & capsule
0.26	/	PP0244		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.26	/	PP4943		glycosyl transferase, putative	Putative enzymes
0.25	/	PP4015		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.25	/	PP1461	*ffh*	signal recognition particle protein Ffh	Protein secretion & export apparatus
0.25	/	PP0702		major facilitator family transporter	Transport of small molecules
0.25	/	PP4802		conserved hypothetical protein	Hypothetical, unclassified, unknown

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
0.24	/	PP0746	*upp*	uracil phosphoribosyltransferase	Nucleotide biosynthesis & metabolism
0.24	/	PP1304	*cysNC*	sulfate adenylyltransferase, subunit 1/adenylylsulfate kinase	Amino acid biosynthesis & metabolism
0.24	/	PP1495		unknown	Hypothetical, unclassified, unknown
0.24	0.49	PP0834	*yajC*	preprotein translocase, YajC subunit	Protein secretion & export apparatus
0.24	0.50	PP1609		hypothetical protein	Hypothetical, unclassified, unknown
0.24	/	PP1177	*nrdB*	ribonucleoside reductase, beta subunit	Nucleotide biosynthesis & metabolism
0.24	/	PP0842	*iscS-1*	cysteine desulfurase	Amino acid biosynthesis & metabolism
0.24	/	PP0815	*cyoD*	cytochrome o ubiquinol oxidase, protein CyoD	Energy metabolism
0.23	/	PP1088	*argG*	argininosuccinate synthase	Amino acid biosynthesis & metabolism
0.23	0.47	PP0832	*queA*	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	Translation, post-translational modification & degradation
0.23	/	PP1213	*aspS*	aspartyl-tRNA synthetase	Translation, post-translational modification & degradation
0.23	/	PP3921		hypothetical protein	Hypothetical, unclassified, unknown
0.23	/	PP0864		ornithine decarboxylase, putative	Amino acid biosynthesis & metabolism
0.23	0.51	PP1782	*rmlC*	dTDP-4-dehydrorhamnose 3,5-epimerase	Cell wall, LPS & capsule
0.23	/	PP0058		1-acyl-sn-glycerol-3-phosphate acyltransferase, putative	Fatty acid & phospholipid metabolism
0.23	0.52	PP1642		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.23	0.48	PP0836	*secF*	protein-export membrane protein SecF	Protein secretion & export apparatus
0.23	/	PP1239		metallo-beta-lactamase family protein	Putative enzymes
0.23	/	PP1791		aldolase/synthase, putative	Putative enzymes
0.23	/	PP2099		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.23	0.49	PP1786		glycosyl transferase, putative	Putative enzymes
0.22	0.54	PP1601	*lpxD*	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	Cell wall, LPS & capsule
0.22	/	PP1996	*accD*	acetyl-CoA carboxylase, carboxyl transferase, beta subunit	Fatty acid & phospholipid metabolism
0.22	0.45	PP2929	*nspC*	carboxynorspermidine decarboxylase	Amino acid biosynthesis & metabolism
0.22	/	PP0813	*cyoB*	cytochrome o ubiquinol oxidase, subunit I	Energy metabolism
0.22	0.50	PP1431	*lepA*	GTP-binding protein LepA	Protein secretion & export apparatus
0.22	0.46	PP0674		ABC transporter, ATP-binding protein	Transport of small molecules
0.22	/	PP4965	*tktA*	transketolase	Energy metabolism
0.22	/	PP4018		acetyltransferase, GNAT family	Putative enzymes

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
0.21	0.46	PP1605	*rnhB*	ribonuclease HII	DNA replication, recombination, modification & repair
0.21	0.46	PP1245		hypothetical protein	Hypothetical, unclassified, unknown
0.21	0.53	PP0841		BadM/Rrf2 family transcriptional regulator	Transcriptional regulators
0.21	/	PP1594	*frr*	ribosome recycling factor	Translation, post-translational modification & degradation
0.21	0.53	PP0560	*aroQ-1*	3-dehydroquinate dehydratase, type II	Amino acid biosynthesis & metabolism
0.21	/	PP0816	*cyoE-2*	protoheme IX farnesyltransferase	Energy metabolism
0.21	0.47	PP1596	*cdsA*	phosphatidate cytidylyltransferase	Fatty acid & phospholipid metabolism
0.21	/	PP1197		conserved hypothetical protein TIGR01125	Hypothetical, unclassified, unknown
0.21	0.51	PP4962		hypothetical protein	Hypothetical, unclassified, unknown
0.21	/	PP4977	*metF*	5,10-methylenetetrahydrofolate reductase	Amino acid biosynthesis & metabolism
0.21	/	PP0933	*mreB*	rod shape-determining protein MreB	Cell division
0.21	/	PP1595	*uppS*	undecaprenyl diphosphate synthase	Cell wall, LPS & capsule
0.20	/	PP0519	*thiL*	thiamine monophosphate kinase	Biosynthesis of cofactors, prosthetic groups & carriers
0.20	0.44	PP1783	*rmlA*	glucose-1-phosphate thymidylyltransferase	Cell wall, LPS & capsule
0.20	/	PP1822		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.20	0.47	PP1224		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.20	0.51	PP1790		acylneuraminate cytidylyltransferase, putative	Putative enzymes
0.20	/	PP0838	*suhB*	extragenic suppressor protein SuhB	Translation, post-translational modification & degradation
0.19	0.42	PP1433	*rnc*	ribonuclease III	Transcription, RNA processing & degradation
0.19	0.51	PP1625		ferrodoxin, 4Fe-4S	Energy metabolism
0.19	0.50	PP1612	*eno*	enolase	Carbon compound catabolism
0.19	0.38	PP0848		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.19	0.41	PP4808		conserved hypothetical protein TIGR00246	Hypothetical, unclassified, unknown
0.19	0.46	PP1665	*purM*	phosphoribosylformylglycinamidine cyclo-ligase	Nucleotide biosynthesis & metabolism
0.19	0.40	PP4709	*rpsO*	ribosomal protein S15	Translation, post-translational modification & degradation
0.18	/	PP1916	*fabF*	3-oxoacyl-(acyl-carrier-protein) synthase II	Fatty acid & phospholipid metabolism
0.18	0.39	PP4017		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.18	/	PP0812	*cyoA*	cytochrome o ubiquinol oxidase, subunit II	Energy metabolism
0.18	0.41	PP0440	*tuf-1*	translation elongation factor Tu	Translation, post-translational modification & degradation

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
0.18	/	PP0811	*cyoups2*	cyoups2 protein	Hypothetical, unclassified, unknown
0.18	/	PP4807	*mrdA-2*	penicillin-binding protein 2	Cell wall, LPS & capsule
0.17	0.38	PP1249		hypothetical protein	Hypothetical, unclassified, unknown
0.17	0.45	PP1611	*kdsA-1*	2-dehydro-3-deoxyphosphooctonate aldolase	Cell wall, LPS & capsule
0.16	0.36	PP0835	*secD*	protein-export membrane protein SecD	Protein secretion & export apparatus
0.16	0.40	PP1432	*lepB*	signal peptidase I	Protein secretion & export apparatus
0.16	/	PP1496	*lysS*	lysyl-tRNA synthetase	Translation, post-translational modification & degradation
0.15	0.36	PP1434	*era*	GTP-binding protein Era	Translation, post-translational modification & degradation
0.15	/	PP1214		conserved hypothetical protein TIGR01033	Hypothetical, unclassified, unknown
0.15	0.41	PP5335	*purK*	phosphoribosylaminoimidazole carboxylase, ATPase subunit	Nucleotide biosynthesis & metabolism
0.15	0.30	PP5413	*atpD*	ATP synthase F1, beta subunit	Energy metabolism
0.15	0.44	PP5336	*purE*	phosphoribosylaminoimidazole carboxylase, catalytic subunit	Nucleotide biosynthesis & metabolism
0.15	0.35	PP0559	*accB*	acetyl-CoA carboxylase, biotin carboxyl carrier protein	Fatty acid & phospholipid metabolism
0.15	0.39	PP0448	*rpoC*	DNA-directed RNA polymerase, beta' subunit	Transcription, RNA processing & degradation
0.14	0.32	PP0478	*rpsD*	ribosomal protein S4	Translation, post-translational modification & degradation
0.14	0.44	PP0441	*secE*	preprotein translocase, SecE subunit	Protein secretion & export apparatus
0.14	0.37	PP1592	*tsf*	translation elongation factor Ts	Translation, post-translational modification & degradation
0.14	0.33	PP4016	*purB*	adenylosuccinate lyase	Nucleotide biosynthesis & metabolism
0.13	0.32	PP5412	*atpC*	ATP synthase F1, epsilon subunit	Energy metabolism
0.13	0.26	PP4714		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.13	0.30	PP1593	*pyrH*	uridylate kinase	Nucleotide biosynthesis & metabolism
0.13	0.33	PP1789		hydrolase, haloacid dehalogenase-like family	Putative enzymes
0.13	0.33	PP1771	*cmk*	cytidylate kinase	Nucleotide biosynthesis & metabolism
0.13	0.30	PP4711	*rbfA*	ribosome-binding factor A	Translation, post-translational modification & degradation
0.13	0.29	PP5265	*xpt*	xanthine phosphoribosyltransferase	Nucleotide biosynthesis & metabolism
0.13	0.38	PP0720	*pth*	peptidyl-tRNA hydrolase	Translation, post-translational modification & degradation
0.12	0.33	PP4710		unknown	Hypothetical, unclassified, unknown
0.12	0.29	PP4712	*infB*	translation initiation factor IF-2	Translation, post-translational modification & degradation
0.12	0.26	PP0477	*rpsK*	ribosomal protein S11	Translation, post-translational modification & degradation

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
0.11	0.35	PP0443	*rplK*	ribosomal protein L11	Translation, post-translational modification & degradation
0.11	0.25	PP0008	*rnpA*	ribonuclease P protein component	Translation, post-translational modification & degradation
0.11	0.26	PP0480	*rplQ*	ribosomal protein L17	Translation, post-translational modification & degradation
0.11	0.29	PP0721		ribosomal 5S rRNA E-loop binding protein Ctc/L25/TL5	Translation, post-translational modification & degradation
0.11	0.29	PP1463	*rimM*	16S rRNA processing protein RimM	Translation, post-translational modification & degradation
0.11	0.23	PP0690		GTP-binding protein, GTP1/Obg family	Adaptation & Protection
0.11	0.24	PP0462	*rpmC*	ribosomal protein L29	Translation, post-translational modification & degradation
0.11	0.25	PP0722	*prsA*	ribose-phosphate pyrophosphokinase	Nucleotide biosynthesis & metabolism
0.11	0.31	PP5415	*atpA*	ATP synthase F1, alpha subunit	Energy metabolism
0.11	0.23	PP0479	*rpoA*	DNA-directed RNA polymerase, alpha subunit	Transcription, RNA processing & degradation
0.11	0.23	PP0469	*rplF*	ribosomal protein L6	Translation, post-translational modification & degradation
0.11	0.38	PP0442	*nusG*	transcription antitermination protein NusG	Transcription, RNA processing & degradation
0.10	0.22	PP1462	*rpsP*	ribosomal protein S16	Translation, post-translational modification & degradation
0.10	0.25	PP5416	*atpH*	ATP synthase F1, delta subunit	Energy metabolism
0.10	0.35	PP0719		GTP-dependent nucleic acid-binding protein EngD	Translation, post-translational modification & degradation
0.10	0.30	PP1316	*rpsI*	ribosomal protein S9	Translation, post-translational modification & degradation
0.10	0.30	PP5417	*atpF*	ATP synthase F0, B subunit	Energy metabolism
0.10	0.26	PP5418	*atpE*	ATP synthase F0, C subunit	Energy metabolism
0.10	0.24	PP5414	*atpG*	ATP synthase F1, gamma subunit	Energy metabolism
0.10	0.20	PP4875		conserved hypothetical protein	Hypothetical, unclassified, unknown
0.10	0.23	PP0444	*rplA*	ribosomal protein L1	Translation, post-translational modification & degradation
0.09	0.21	PP4876	*rpsR*	ribosomal protein S18	Translation, post-translational modification & degradation
0.09	0.23	PP2468	*rplT*	ribosomal protein L20	Translation, post-translational modification & degradation
0.09	0.23	PP4713	*nusA*	N utilization substance protein A	Transcription, RNA processing & degradation
0.08	0.20	PP0689	*rpmA*	ribosomal protein L27	Translation, post-translational modification & degradation
0.08	0.19	PP5087	*rpmE*	ribosomal protein L31	Translation, post-translational modification & degradation
0.07	0.26	PP0447	*rpoB*	DNA-directed RNA polymerase, beta subunit	Transcription, RNA processing & degradation
0.07	0.23	PP4877	*rpsF*	ribosomal protein S6	Translation, post-translational modification & degradation
0.07	0.21	PP0476	*rpsM*	ribosomal protein S13	Translation, post-translational modification & degradation

FC wt anaerobic	FC Δr/Δs anaerobic	PP number	gene name	function of gene product	functional classification
0.07	0.21	PP0474	*secY*	Sec-dependent secretion protein SecY	Protein secretion & export apparatus
0.07	0.17	PP4874	*rplI*	ribosomal protein L9	Translation, post-translational modification & degradation
0.06	0.21	PP0688	*rplU*	ribosomal protein L21	Translation, post-translational modification & degradation
0.06	0.13	PP0600	*rpsT*	ribosomal protein S20	Translation, post-translational modification & degradation
0.06	0.20	PP1772	*rpsA*	ribosomal protein S1	Translation, post-translational modification & degradation
0.06	0.16	PP1591	*rpsB*	ribosomal protein S2	Translation, post-translational modification & degradation
0.06	0.16	PP0456	*rplW*	ribosomal protein L23	Translation, post-translational modification & degradation
0.06	0.20	PP0470	*rplR*	ribosomal protein L18	Translation, post-translational modification & degradation
0.06	0.13	PP0455	*rplD*	ribosomal protein L4	Translation, post-translational modification & degradation
0.05	0.13	PP0458	*rpsS*	ribosomal protein S19	Translation, post-translational modification & degradation
0.05	0.13	PP0459	*rplV*	ribosomal protein L22	Translation, post-translational modification & degradation

Appendix I

***In silico* identified genes encoded in the genome of facultative anaerobic but not obligate aerobic *Pseudomonas* strains by "Comparative Genome Search" with "*Pseudomonas* Genome Database".** Genomes of facultative anaerobic strains *P. aeruginosa* PAO1 and *P. stutzeri* A1501 were compared to those of obligate anaerobic strains *P. putida*, *P. fluorescens, P. mendocina, P. syringae* and *P. entomophila.* Identified genes which are not located within the denitrification gene clusters, were categorized in four classes based on the available information content of the respective gene product according to "*Pseudomonas* Genome Database" (2.10.4). Genes are sorted by their appointed classes, descriptions of encoded proteins are according to "*Pseudomonas* Genome Database".

PA/PST number	gene name	function of gene product	class
PA4555	*pilY2*	type 4 fimbrial biogenesis protein PilY2	1
PA5276	*lppL*	Lipopeptide LppL precursor	1
PA5496	*nrdJb*	class II (cobalamin-dependent) ribonucleotide-diphosphate reductase subunit	1
PA5497	*nrdJa*	class II (cobalamin-dependent) ribonucleotide-diphosphate reductase subunit	1
PST0109		ribonucleotide reductase	1
PST0110		ribonucleotide reductase	1
PST0969	*pilY2*	type 4 fimbrial biogenesis protein PilY2	1
PST1243	*putP-1*	sodium/proline symporter PutP	1
PST3384	*dipZ*	thiol:disulfide interchange protein precursor	1
PA1124	*dgt*	deoxyguanosinetriphosphate triphosphohydrolase	2
PA1919	*nrdG*	class III (anaerobic) ribonucleoside-triphosphate reductase activating protein	2
PA1920	*nrdD*	class III (anaerobic) ribonucleoside-triphosphate reductase subunit	2
PA2416	*treA*	periplasmic trehalase precursor	2
PST1588	*dgt*	deoxyguanosinetriphosphate triphosphohydrolase	2
PST2159		formate dehydrogenase-O	2
PST3507		radical-activating enzyme	2
PST3509		anaerobic ribonucleoside triphosphate reductase	2
PST3829		InaA protein	2
PST3894	*treA*	periplasmic trehalase precursor	2
PA0172		hypothetical protein	3

PA/PST number	gene name	function of gene product	class
PA0249		probable acetyltransferase	3
PA0334		probable major facilitator superfamily (MFS) transporter	3
PA2533		probable sodium:alanine symporter	3
PA2694		probable thioredoxin	3
PA2788		probable chemotaxis transducer	3
PA3718		probable major facilitator superfamily (MFS) transporter	3
PA3913		probable protease	3
PA3914	*moeA1*	molybdenum cofactor biosynthetic protein A1	3
PA4887		probable major facilitator superfamily (MFS) transporter	3
PA5313		probable pyridoxal-dependent aminotransferase	3
PA5514		probable beta-lactamase	3
PST0158		glycine cleavage system H protein	3
PST0407		MFS family transporter	3
PST0927		putative acyltransferase	3
PST0944		putative copper export protein	3
PST1289		TonB-dependent receptor	3
PST1597		methyl-accepting chemotaxis transducer	3
PST1802		MFS family transporter	3
PST2182		sodium:alanine symporter	3
PST2699		beta-lactamase	3
PST2733		Zn-dependent hydrolase	3
PST2836		thioredoxin	3
PST3512		collagenase	3
PST3513		protease	3
PST3556		c4-dicarboxylate-binding protein	3
PST4021		DMT family permease	3
PST4095		putative acetyltransferase	3
PA0170		hypothetical protein	4
PA0171		hypothetical protein	4

PA/PST number	gene name	function of gene product	class
PA1404		hypothetical protein	4
PA1451		conserved hypothetical protein	4
PA1577		hypothetical protein	4
PA1851		hypothetical protein	4
PA1923		hypothetical protein	4
PA1924		hypothetical protein	4
PA1925		hypothetical protein	4
PA2753		hypothetical protein	4
PA3631		conserved hypothetical protein	4
PA3632		conserved hypothetical protein	4
PA3912		conserved hypothetical protein	4
PA4338		hypothetical protein	4
PA4610		hypothetical protein	4
PA4795		hypothetical protein	4
PA4796		hypothetical protein	4
PA4962		conserved hypothetical protein	4
PA5144		hypothetical protein	4
PA5250		conserved hypothetical protein	4
PST0157		hypothetical protein	4
PST0620		hypothetical protein	4
PST0831		hypothetical protein	4
PST0935		hypothetical protein	4
PST0936		hypothetical protein	4
PST0937		hypothetical protein	4
PST0938		hypothetical protein	4
PST1451		hypothetical protein	4
PST1586		hypothetical protein	4
PST1794		hypothetical protein	4
PST1795		hypothetical protein	4

PA/PST number	gene name	function of gene product	class
PST1869		hypothetical protein	4
PST2472		hypothetical protein	4
PST2665		hypothetical protein	4
PST3511		hypothetical protein	4

Appendix J

Genes differentially regulated in *Pseudomonas putida* KT2440-NAR expressing the *Pseudomonas aeruginosa narK$_1$K$_2$GHIJLX* operon after 30 minutes of oxygen depletion of mid-exponential phase cultures in the presence of nitrate. Experimental procedures and data analysis are described elsewhere (3.2.3.5). PP and PA numbers, gene names and description of encoded proteins are according to "*Pseudomonas* Genome Database". Enlisted are fold change expression values for KT2440-NAR after 30 minutes of anaerobic incubation in the presence of nitrate compared to KT2440-pLAFR3 prior to anaerobic shift ("FC anaerobic with nitrate") and KT2440-NAR prior to anaerobic shift compared to KT2440-pLAFR3 prior to anaerobic shift ("FC aerobic"). Fold change expression values lower than 1.75-fold and higher than 0.57-fold are not enlisted ("/").

FC anaerobic with nitrate	FC aerobic	PP / PA number	gene name	function of gene product
108.38	5.13	PP5393		heavy metal transport/detoxification protein
46.53	31.34	PP5395		hypothetical protein
40.50	8.28	PP5391		hypothetical protein
10.20	3.61	PP5392		YVTN family beta-propeller repeat-containing protein
9.85	12.73	PA3877	*narK1*	PA nitrite extrusion protein 1
9.78	14.03	PA3874	*narH*	PA respiratory nitrate reductase beta chain
9.19	3.81	PP5389		hypothetical protein
8.63	13.27	PA3876	*narK2*	PA nitrite extrusion protein 2
8.63	3.20	PP5394		hypothetical protein
8.57	12.38	PA3878	*narX*	PA two-component sensor NarX
8.22	11.47	PA3872	*narI*	PA respiratory nitrate reductase gamma chain
7.16	6.68	PP5395.1		unknown
6.82	5.50	PP5400		hypothetical protein
6.50	2.38	PP5390		hypothetical protein
6.19	10.20	PA3875	*narG*	PA respiratory nitrate reductase alpha chain
5.74	8.34	PA3865		probable amino acid binding protein
5.10	4.99	PP5399		hypothetical protein
4.76	7.94	PA3870	*moaA1*	PA molybdopterin biosynthetic protein A1
4.56	4.69	PP5401		hypothetical protein

FC anaerobic with nitrate	FC aerobic	PP / PA number	gene name	function of gene product
3.58	4.69	PA3871		PA probable peptidyl-prolyl cis-trans isomerase, PpiC-type
3.23	/	PP4735	*lctP*	L-lactate transporter
2.73	/	PP1291		PhoH family protein
2.71	/	PP0596		beta alanine--pyruvate transaminase
0.37	0.44	PP3785		hypothetical protein
0.35	0.38	PP3783		hypothetical protein
0.33	/	PP1249		hypothetical protein
0.32	0.42	PP5338	*aspA*	aspartate ammonia-lyase
0.30	0.35	PP3781		oxygen-independent Coproporphyrinogen III oxidase family protein

Appendix K

Genes differentially regulated in *Pseudomonas putida* KT2440-NIR-NOR expressing the *Pseudomonas aeruginosa nirSMCFDLGHJEN-norBCD* operon after 30 minutes of oxygen depletion of mid-exponential phase cultures in the presence of nitrite. Experimental procedures and data analysis are described elsewhere (3.2.3.6). PP and PA numbers, gene names and description of encoded proteins are according to "*Pseudomonas* Genome Database". Enlisted are fold change expression values for KT2440-NIR-NOR after 30 minutes of anaerobic incubation in the presence of nitrite compared to KT2440-pLAFR3 prior to anaerobic shift ("FC anaerobic with nitrite"), KT2440-NIR-NOR prior to anaerobic shift compared to KT2440-pLAFR3 prior to anaerobic shift ("FC aerobic") and KT2440-NIR-NOR after 30 minutes of aerobic incubation with nitrite compared to KT2440-pLAFR3 prior to anaerobic shift ("FC aerobic with nitrite"). Fold change expression values lower than 1.75-fold and higher than 0.57-fold are not enlisted ("/").

FC anaerobic with nitrite	FC aerobic	FC aerobic with nitrite	PP / PA number	gene name	function of gene product
55.72	27.86	31.78	PP5395		hypothetical protein
31.78	4.86	6.15	PP5391		hypothetical protein
23.92	3.61	3.89	PA0523	*norC*	PA nitric-oxide reductase subunit C
16.22	3.14	3.48	PP5393		heavy metal transport/detoxification protein
15.24	3.63	3.41	PA0526		PA hypothetical protein
11.00	3.34	/	PA0524	*norB*	PA nitric-oxide reductase subunit B
10.78	3.46	3.34	PA0519	*nirS*	PA nitrite reductase precursor
8.51	/	2.81	PP5394		hypothetical protein
7.94	4.29	6.06	PP5395.1		unknown
6.82	4.00	3.61	PA0518	*nirM*	PA cytochrome c-551 precursor
6.15	2.07	/	PP5389		hypothetical protein
6.02	7.01	7.01	PP1691		hypothetical protein
5.82	4.17	3.48	PP5400		hypothetical protein
5.31	3.63	4.89	PP5399		hypothetical protein
4.99	2.95	/	PA0520	*nirQ*	PA regulatory protein NirQ
4.86	/	/	PP5390		hypothetical protein
4.69	3.56	3.07	PA0527	*dnr*	PA transcriptional regulator Dnr
4.44	3.56	3.48	PA0521		PA probable cytochrome c oxidase subunit

FC anaerobic with nitrite	FC aerobic	FC aerobic with nitrite	PP / PA number	gene name	function of gene product
4.38	3.81	3.78	PA0511	*nirJ*	PA heme d1 biosynthesis protein NirJ
4.32	3.66	3.39	PA0528		PA probable transcriptional regulator
4.29	3.61	3.29	PA0510		PA probable uroporphyrin-III c-methyltransferase
4.26	3.34	3.63	PP5401		hypothetical protein
4.00	3.92	5.86	PA0529		PA conserved hypothetical protein
3.97	/	/	PP2359		spore coat U domain protein
3.92	2.41	/	PP4496		hypothetical protein
3.92	/	3.41	PP4424		AsnC family transcriptional regulator
3.84	3.23	3.86	PA0530		PA probable class III pyridoxal phosphate-dependent aminotransferase
3.63	3.25	2.97	PA0514	*nirL*	PA heme d1 biosynthesis protein NirL
3.61	3.68	2.91	PA0512		PA conserved hypothetical protein
3.58	3.73	/	PP0445	*rplJ*	50S ribosomal protein L10
3.43	/	4.59	PP3764		outer membrane porin
3.39	3.61	/	PA0516	*nirF*	PA heme d1 biosynthesis protein NirF
3.29	3.2	/	PA0515		PA probable transcriptional regulator
3.23	3.07	2.95	PP2468	*rplT*	50S ribosomal protein L20
3.18	2.95	/	PA0517	*nirC*	PA probable c-type cytochrome precursor
3.12	3.97	2.95	PP2467	*rpmI*	50S ribosomal protein L35
3.07	3.39	3.20	PP4710	*truB*	tRNA pseudouridine synthase B
3.05	3.14	/	PP5392		YVTN family beta-propeller repeat-containing protein
3.05	/	2.93	PP4711	*rbfA*	ribosome-binding factor A
3.03	1.97	/	PP2223		hypothetical protein
2.99	4.72	3.12	PP1868		DEAD-box ATP dependent DNA helicase
2.91	3.56	3.41	PP3684.1		unknown
2.91	/	/	PP2360		spore coat U domain protein
2.91	2.36	/	PP0446	*rplL*	50S ribosomal protein L7/L12
2.89	4.00	/	PP4877	*rpsF*	30S ribosomal protein S6
2.89	3.51	3.81	PP4292		hypothetical protein
2.85	/	/	PP2358		spore coat U domain protein

FC anaerobic with nitrite	FC aerobic	FC aerobic with nitrite	PP / PA number	gene name	function of gene product
2.83	3.86	3.51	PP4293		hypothetical protein
2.77	1.93	5.82	PP2645	*mgtB*	magnesium-translocating P-type ATPase
2.77	/	/	PP1742		hypothetical protein
2.77	2.73	/	PA0522		PA hypothetical protein
2.71	2.01	/	PP2629		hypothetical protein
0.37	0.27	/	PP4221		non-ribosomal peptide synthetase
0.37	0.50	0.21	PP1360	*groES*	co-chaperonin GroES
0.37	0.48	0.30	PP3775		sarcosine oxidase, putative
0.37	0.44	0.37	PP3790	*dapF*	diaminopimelate epimerase
0.37	/	0.34	PP3777		hypothetical protein
0.37	0.39	/	PP4245		siderophore biosynthesis protein, putative
0.36	0.46	/	PP2901		penicillin amidase family protein
0.36	0.43	0.23	PP3782		hypothetical protein
0.36	0.46	0.31	PP4216	*pvdE*	cyclic peptide transporter
0.36	0.36	0.31	PP4243		peptide synthase
0.35	/	0.30	PP4223		diaminobutyrate--2-oxoglutarate aminotransferase
0.34	0.44	0.29	PP0913		hypothetical protein
0.34	0.51	/	PP0947		zinc/iron permease
0.34	0.43	0.31	PP4201		electron transfer flavoprotein, alpha subunit
0.33	0.43	/	PP4214		aminotransferase, class V
0.33	0.46	0.35	PP3796		L-ornithine N5-oxygenase
0.32	0.51	0.27	PP0944	*fumC*	fumarate hydratase
0.31	0.55	0.18	PP3784		hypothetical protein
0.31	/	0.22	PP3787		hypothetical protein
0.31	/	0.23	PP4185	*sucD*	succinyl-CoA synthetase subunit alpha
0.3	0.45	/	PP2900		hypothetical protein
0.3	/	0.21	PP3785		hypothetical protein
0.3	/	0.34	PP4215		hypothetical protein
0.3	0.42	0.28	PP3776	*rarD-3*	rarD protein

FC anaerobic with nitrite	FC aerobic	FC aerobic with nitrite	PP / PA number	gene name	function of gene product
0.29	0.50	0.15	PP1361	*groEL*	chaperonin GroEL
0.26	0.26	0.10	PP2674	*qedH*	quinoprotein ethanol dehydrogenase
0.25	0.35	0.24	PP4222		syrP protein, putative
0.24	0.39	0.21	PP4186	*sucC*	succinyl-CoA synthetase subunit beta
0.23	0.52	/	PP1249		hypothetical protein
0.23	0.35	0.30	PP0946	*sodA*	superoxide dismutase
0.22	0.43	0.29	PP3808		MbtH domain protein
0.21	0.27	0.17	PP2680		aldehyde dehydrogenase family protein
0.17	0.34	0.14	PP3781		oxygen-independent Coproporphyrinogen III oxidase family protein

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